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Introduction class="introduction"

A veterinarian gets ready to clean a sea turtle covered in oil following the Deepwater Horizon oil spill in the Gulf of Mexico in 2010. After the spill, the population of a naturally occurring oil-eating marine bacterium called *Alcanivorax borkumensis* skyrocketed, helping to get rid of the oil. Scientists are working on ways to genetically

engineer
this
bacterium to
be more
efficient in
cleaning up
future spills.

(credit:
modification
of work
by NOAA's
National
Ocean
Service)



From boiling thermal hot springs to deep beneath the Antarctic ice, microorganisms can be found almost everywhere on earth in great quantities. Microorganisms (or microbes, as they are also called) are small organisms. Most are so small that they cannot be seen without a microscope.

Most microorganisms are harmless to humans and, in fact, many are helpful. They play fundamental roles in ecosystems everywhere on earth,

forming the backbone of many food webs. People use them to make biofuels, medicines, and even foods. Without microbes, there would be no bread, cheese, or beer. Our bodies are filled with microbes, and our skin alone is home to trillions of them.[\[footnote\]](#) Some of them we can't live without; others cause diseases that can make us sick or even kill us.

J. Hulcr et al. "A Jungle in There: Bacteria in Belly Buttons are Highly Diverse, but Predictable." *PLoS ONE* 7 no. 11 (2012): e47712.
doi:10.1371/journal.pone.0047712.

Although much more is known today about microbial life than ever before, the vast majority of this invisible world remains unexplored.

Microbiologists continue to identify new ways that microbes benefit and threaten humans.

What Our Ancestors Knew

LEARNING OBJECTIVES

- Describe how our ancestors improved food with the use of invisible microbes
- Describe how the causes of sickness and disease were explained in ancient times, prior to the invention of the microscope
- Describe key historical events associated with the birth of microbiology

Most people today, even those who know very little about microbiology, are familiar with the concept of microbes, or “germs,” and their role in human health. Schoolchildren learn about bacteria, viruses, and other microorganisms, and many even view specimens under a microscope. But a few hundred years ago, before the invention of the microscope, the existence of many types of microbes was impossible to prove. By definition, **microorganisms**, or **microbes**, are very small organisms; many types of microbes are too small to see without a microscope, although some parasites and fungi are visible to the naked eye.

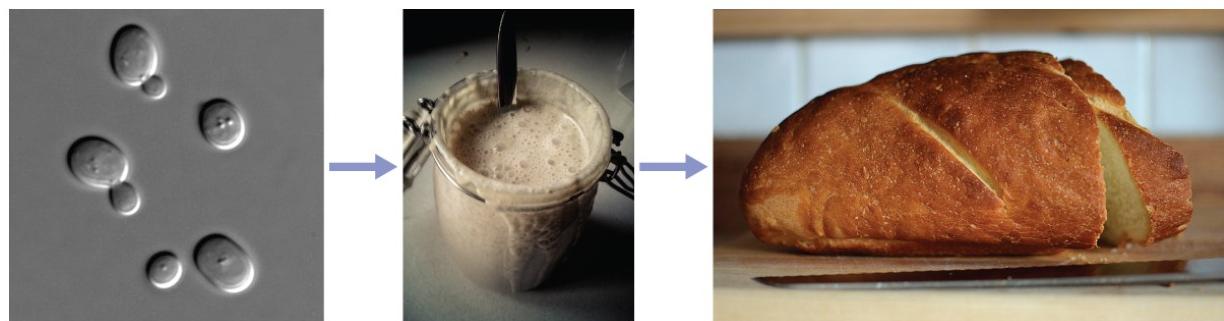
Humans have been living with—and using—microorganisms for much longer than they have been able to see them. Historical evidence suggests that humans have had some notion of microbial life since prehistoric times and have used that knowledge to develop foods as well as prevent and treat disease. In this section, we will explore some of the historical applications of microbiology as well as the early beginnings of microbiology as a science.

Fermented Foods and Beverages

People across the world have enjoyed fermented foods and beverages like beer, wine, bread, yogurt, cheese, and pickled vegetables for all of recorded history. Discoveries from several archeological sites suggest that even prehistoric people took advantage of fermentation to preserve and enhance the taste of food. Archaeologists studying pottery jars from a Neolithic village in China found that people were making a fermented beverage from rice, honey, and fruit as early as 7000 BC.[\[footnote\]](#)

P.E. McGovern et al. “Fermented Beverages of Pre- and Proto-Historic China.” *Proceedings of the National Academy of Sciences of the United States of America* 1 no. 51 (2004):17593–17598.
doi:10.1073/pnas.0407921102.

Production of these foods and beverages requires microbial fermentation, a process that uses bacteria, mold, or yeast to convert sugars (carbohydrates) to alcohol, gases, and organic acids ([\[link\]](#)). While it is likely that people first learned about fermentation by accident—perhaps by drinking old milk that had curdled or old grape juice that had fermented—they later learned to harness the power of fermentation to make products like bread, cheese, and wine.



Yeast fermentation yields ethanol and CO₂.

A microscopic view of *Saccharomyces cerevisiae*, the yeast responsible for making bread rise (left). Yeast is a microorganism. Its cells metabolize the carbohydrates in flour (middle) and produce carbon dioxide, which causes the bread to rise (right). (credit middle:

modification of work by Janus Sandsgaard; credit right: modification of work by “MDreibelbis”/Flickr)

The Iceman Treateth

Prehistoric humans had a very limited understanding of the causes of disease, and various cultures developed different beliefs and explanations. While many believed that illness was punishment for angering the gods or was simply the result of fate, archaeological evidence suggests that prehistoric people attempted to treat illnesses and infections. One example of this is Ötzi the Iceman, a 5300-year-old mummy found frozen in the ice of the Ötztal Alps on the Austrian-Italian border in 1991. Because Ötzi was so well preserved by the ice, researchers discovered that he was infected with the eggs of the parasite *Trichuris trichiura*, which may have caused him to have abdominal pain and anemia. Researchers also found evidence of *Borrelia burgdorferi*, a bacterium that causes Lyme disease.[\[footnote\]](#) Some researchers think Ötzi may have been trying to treat his infections with the woody fruit of the *Piptoporus betulinus* fungus, which was discovered tied to his belongings.[\[footnote\]](#) This fungus has both laxative and antibiotic properties. Ötzi was also covered in tattoos that were made by cutting incisions into his skin, filling them with herbs, and then burning the herbs.[\[footnote\]](#) There is speculation that this may have been another attempt to treat his health ailments.

A. Keller et al. “New Insights into the Tyrolean Iceman's Origin and Phenotype as Inferred by Whole-Genome Sequencing.” *Nature Communications*, 3 (2012): 698. doi:10.1038/ncomms1701.

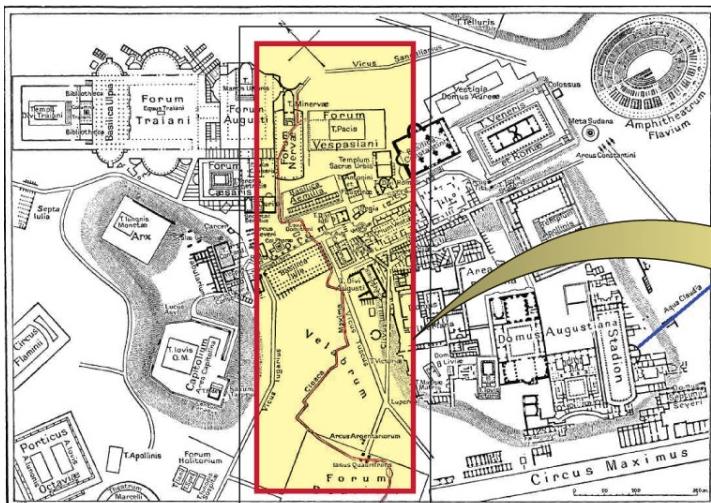
L. Capasso. “5300 Years Ago, the Ice Man Used Natural Laxatives and Antibiotics.” *The Lancet*, 352 (1998) 9143: 1864. doi: 10.1016/s0140-6736(05)79939-6.

L. Capasso, L. “5300 Years Ago, the Ice Man Used Natural Laxatives and Antibiotics.” *The Lancet*, 352 no. 9143 (1998): 1864. doi: 10.1016/s0140-6736(05)79939-6.

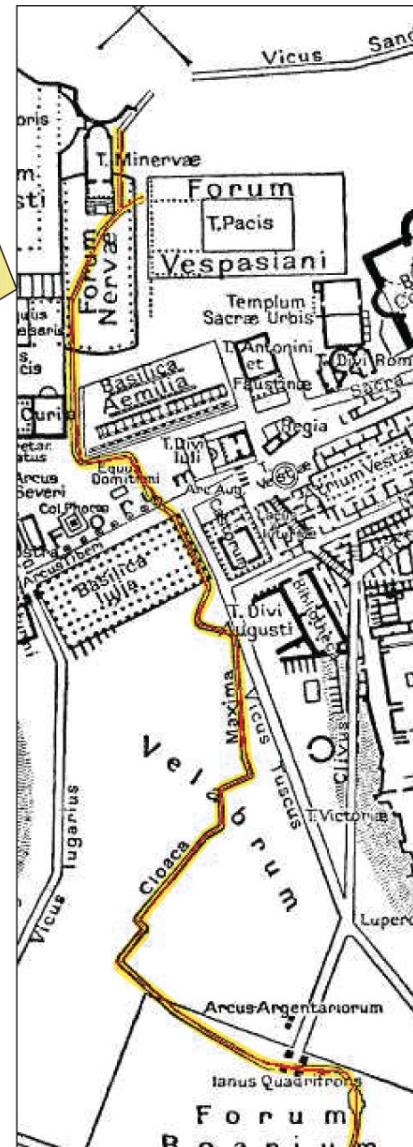
Early Notions of Disease, Contagion, and Containment

Several ancient civilizations appear to have had some understanding that disease could be transmitted by things they could not see. This is especially evident in historical attempts to contain the spread of disease. For example, the Bible refers to the practice of quarantining people with leprosy and other diseases, suggesting that people understood that diseases could be communicable. Ironically, while leprosy is communicable, it is also a disease that progresses slowly. This means that people were likely quarantined after they had already spread the disease to others.

The ancient Greeks attributed disease to bad air, *mal'aria*, which they called “miasmatic odors.” They developed hygiene practices that built on this idea. The Romans also believed in the miasma hypothesis and created a complex sanitation infrastructure to deal with sewage. In Rome, they built aqueducts, which brought fresh water into the city, and a giant sewer, the *Cloaca Maxima*, which carried waste away and into the river Tiber ([\[link\]](#)). Some researchers believe that this infrastructure helped protect the Romans from epidemics of waterborne illnesses.



(a)



(b)

(a) The *Cloaca Maxima*, or “Greatest Sewer” (shown in red), ran through ancient Rome. It was an engineering marvel that carried waste away from the city and into the river Tiber. (b) These ancient latrines emptied into the *Cloaca Maxima*.

Even before the invention of the microscope, some doctors, philosophers, and scientists made great strides in understanding the invisible forces—

what we now know as microbes—that can cause infection, disease, and death.

The Greek physician Hippocrates (460–370 BC) is considered the “father of Western medicine” ([\[link\]](#)). Unlike many of his ancestors and contemporaries, he dismissed the idea that disease was caused by supernatural forces. Instead, he posited that diseases had natural causes from within patients or their environments. Hippocrates and his heirs are believed to have written the *Hippocratic Corpus*, a collection of texts that make up some of the oldest surviving medical books.[\[footnote\]](#) Hippocrates is also often credited as the author of the Hippocratic Oath, taken by new physicians to pledge their dedication to diagnosing and treating patients without causing harm.

G. Pappas et al. “Insights Into Infectious Disease in the Era of Hippocrates.” *International Journal of Infectious Diseases* 12 (2008) 4:347–350. doi: <http://dx.doi.org/10.1016/j.ijid.2007.11.003>.

While Hippocrates is considered the father of Western medicine, the Greek philosopher and historian Thucydides (460–395 BC) is considered the father of scientific history because he advocated for evidence-based analysis of cause-and-effect reasoning ([\[link\]](#)). Among his most important contributions are his observations regarding the Athenian plague that killed one-third of the population of Athens between 430 and 410 BC. Having survived the epidemic himself, Thucydides made the important observation that survivors did not get re-infected with the disease, even when taking care of actively sick people.[\[footnote\]](#) This observation shows an early understanding of the concept of immunity.

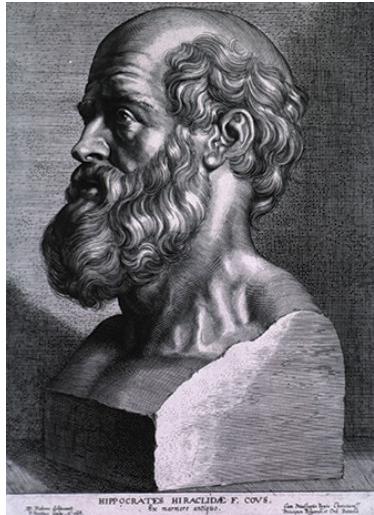
Thucydides. *The History of the Peloponnesian War. The Second Book*. 431 BC. Translated by Richard Crawley.

<http://classics.mit.edu/Thucydides/pelopwar.2.second.html>.

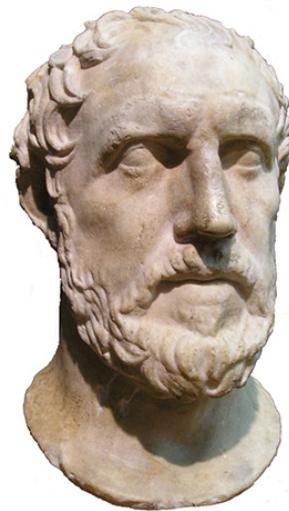
Marcus Terentius Varro (116–27 BC) was a prolific Roman writer who was one of the first people to propose the concept that things we cannot see (what we now call microorganisms) can cause disease ([\[link\]](#)). In *Res Rusticae (On Farming)*, published in 36 BC, he said that “precautions must also be taken in neighborhood swamps . . . because certain minute creatures [*animalia minuta*] grow there which

cannot be seen by the eye, which float in the air and enter the body through the mouth and nose and there cause serious diseases.”[\[footnote\]](#)

Plinio Prioreschi. *A History of Medicine: Roman Medicine*. Lewiston, NY: Edwin Mellen Press, 1998: p. 215.



(a)



(b)



(c)

(a) Hippocrates, the “father of Western medicine,” believed that diseases had natural, not supernatural, causes. (b) The historian Thucydides observed that survivors of the Athenian plague were subsequently immune to the infection. (c) Marcus Terentius Varro proposed that disease could be caused by “certain minute creatures . . . which cannot be seen by the eye.” (credit c: modification of work by Alessandro Antonelli)

Note:

- Give two examples of foods that have historically been produced by humans with the aid of microbes.

- Explain how historical understandings of disease contributed to attempts to treat and contain disease.

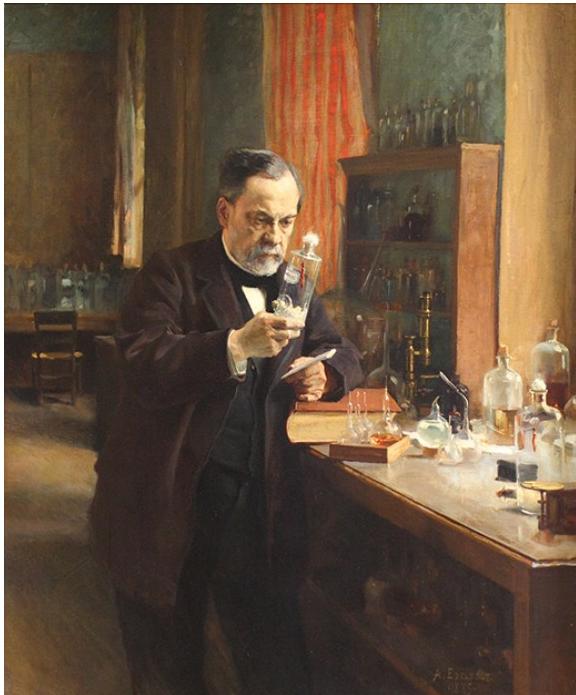
The Birth of Microbiology

While the ancients may have suspected the existence of invisible “minute creatures,” it wasn’t until the invention of the microscope that their existence was definitively confirmed. While it is unclear who exactly invented the microscope, a Dutch cloth merchant named Antonie van Leeuwenhoek (1632–1723) was the first to develop a lens powerful enough to view microbes. In 1675, using a simple but powerful microscope, Leeuwenhoek was able to observe single-celled organisms, which he described as “animalcules” or “wee little beasties,” swimming in a drop of rain water. From his drawings of these little organisms, we now know he was looking at bacteria and protists. (We will explore Leeuwenhoek’s contributions to microscopy further in [How We See the Invisible World](#).)

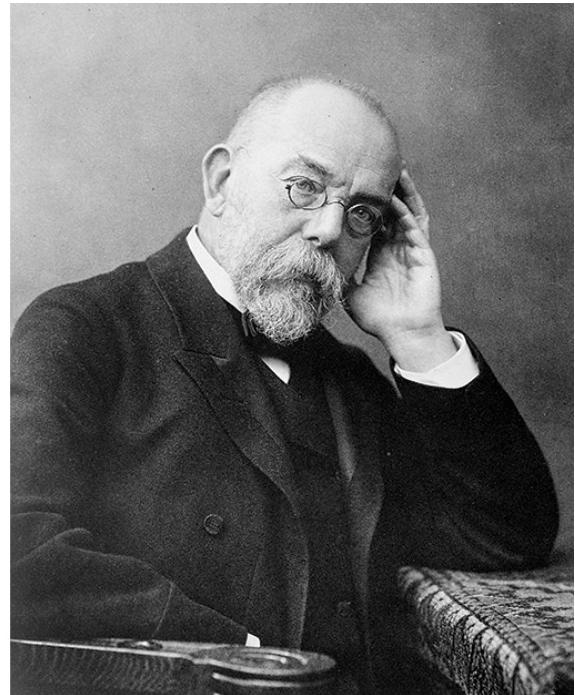
Nearly 200 years after van Leeuwenhoek got his first glimpse of microbes, the “Golden Age of Microbiology” spawned a host of new discoveries between 1857 and 1914. Two famous microbiologists, Louis Pasteur and Robert Koch, were especially active in advancing our understanding of the unseen world of microbes ([\[link\]](#)). Pasteur, a French chemist, showed that individual microbial strains had unique properties and demonstrated that fermentation is caused by microorganisms. He also invented pasteurization, a process used to kill microorganisms responsible for spoilage, and developed vaccines for the treatment of diseases, including rabies, in animals and humans. Koch, a German physician, was the first to demonstrate the connection between a single, isolated microbe and a known human disease. For example, he discovered the bacteria that cause anthrax (*Bacillus anthracis*), cholera (*Vibrio cholera*), and tuberculosis (*Mycobacterium tuberculosis*).[\[footnote\]](#) We will discuss these famous microbiologists, and others, in later chapters.

S.M. Blevins and M.S. Bronze. “Robert Koch and the ‘Golden Age’ of Bacteriology.” *International Journal of Infectious Diseases*. 14 no. 9 (2010): e744-e751. doi:10.1016/j.ijid.2009.12.003.

The discoveries made by Pasteur and Koch led Joseph Lister, a British surgeon, to propose much needed sanitation procedures and aseptic surgical methods. Have in mind that it was common practice not to wash hands or instruments to attend patients. Lister started using carbolic acid, a phenol compound, to clean wounds, soak bandages and to sterilize surgical instruments. A sharp decrease in the risk of contamination was observed when to Lister's methods were applied. He is now considered the "Father of Modern Surgery".



(a)



(b)

(a) Louis Pasteur (1822–1895) is credited with numerous innovations that advanced the fields of microbiology and immunology. (b) Robert Koch (1843–1910) identified the specific microbes that cause anthrax, cholera, and tuberculosis.

As the field of microbiology has developed, it has diversified into many specialized subfields of microbiology:

- **Bacteriology:** the study of bacteria
- **Virology:** the study of viruses
- **Mycology:** the study of fungi
- **Parasitology:** the study of parasites
- **Phycology:** the study of algae
- **Applied microbiology:** the application of microbes to benefit the environment, human and animal health, agriculture, and industry
- **Medical microbiology:** the study of the pathogenic microbes and the role of microbes in human illness
- **Industrial microbiology:** the application of microbes for use in industrial processes
- **Biotechnology:** the manipulation of microbes at the genetic and molecular level to generate useful products
- **Food microbiology:** the study of microbes causing food spoilage and foodborne illness
- **Plant microbiology/pathology:** The study of the interactions between microbes, plants and plant pathogens
- **Soil microbiology:** the study of those microbes that are found in soil
- **Veterinary microbiology:** the study of the role of microbes in veterinary medicine
- **Environmental microbiology:** the study of the function and diversity of microbes in their natural environments
- **Aquatic microbiology:** The study of those microbes that are found in water
- **Air microbiology:** The study of airborne microbes
- and many others

Advancements and discoveries in microbiology has also allowed the broader discipline of biology to grow and flourish in previously unimagined ways. Much of what we know about human cells comes from our understanding of microbes, and many of the tools we use today to study cells and their genetics derive from work with microbes.

Note:

- How did the discovery of microbes change human understanding of disease?

Note:

Microbiology Toolbox

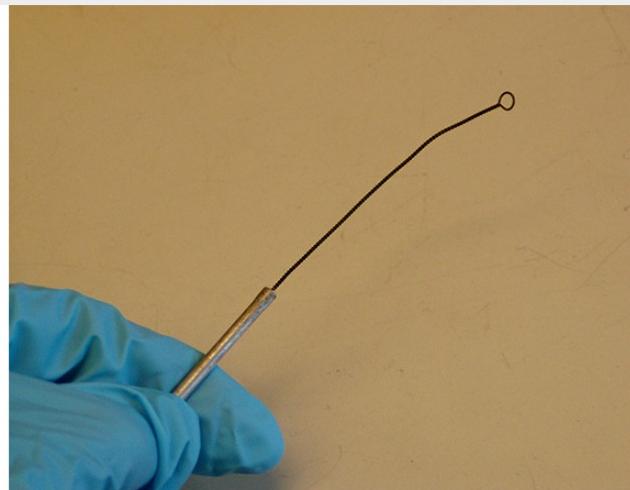
Because individual microbes are generally too small to be seen with the naked eye, the science of microbiology is dependent on technology that can artificially enhance the capacity of our natural senses of perception. Early microbiologists like Pasteur and Koch had fewer tools at their disposal than are found in modern laboratories, making their discoveries and innovations that much more impressive. Later chapters of this text will explore many applications of technology in depth, but for now, here is a brief overview of some of the fundamental tools of the microbiology lab.

- **Microscopes** produce magnified images of microorganisms, human cells and tissues, and many other types of specimens too small to be observed with the naked eye.
- **Stains and dyes** are used to add color to microbes so they can be better observed under a microscope. Some dyes can be used on living microbes, whereas others require that the specimens be fixed with chemicals or heat before staining. Some stains only work on certain types of microbes because of differences in their cellular chemical composition.
- **Growth media** are used to grow microorganisms in a lab setting. Some media are liquids; others are more solid or gel-like. A growth medium provides nutrients, including water, various salts, a source of carbon (like glucose), and a source of nitrogen and amino acids (like yeast extract) so microorganisms can grow and reproduce. Ingredients in a growth medium can be modified to grow unique types of microorganisms.
- **A Petri dish** is a flat-lidded dish that is typically 10–11 centimeters (cm) in diameter and 1–1.5 cm high. Petri dishes made out of either plastic or glass are used to hold growth media ([\[link\]](#)).

- **Test tubes** are cylindrical plastic or glass tubes with rounded bottoms and open tops. They can be used to grow microbes in broth, or semisolid or solid growth media.
- A **Bunsen burner** is a metal apparatus that creates a flame that can be used to sterilize pieces of equipment. A rubber tube carries gas (fuel) to the burner. In many labs, Bunsen burners are being phased out in favor of infrared **microincinerators**, which serve a similar purpose without the safety risks of an open flame.
- An **inoculation loop** is a handheld tool that ends in a small wire loop ([\[link\]](#)). The loop can be used to streak microorganisms on agar in a Petri dish or to transfer them from one test tube to another. Before each use, the inoculation loop must be sterilized so cultures do not become contaminated.



(a)



(b)

(a) This Petri dish filled with agar has been streaked with *Legionella*, the bacterium responsible for causing Legionnaire's disease. (b) An inoculation loop like this one can be used to streak bacteria on agar in a Petri dish. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by Jeffrey M. Vinocur)

Key Concepts and Summary

- **Microorganisms** (or **microbes**) are living organisms that are generally too small to be seen without a microscope.
- Throughout history, humans have used microbes to make fermented foods such as beer, bread, cheese, and wine.
- Long before the invention of the microscope, some people theorized that infection and disease were spread by living things that were too small to be seen. They also correctly intuited certain principles regarding the spread of disease and immunity.
- Antonie van Leeuwenhoek, using a microscope, was the first to actually describe observations of bacteria, in 1675.
- During the Golden Age of Microbiology (1857–1914), microbiologists, including Louis Pasteur and Robert Koch, discovered many new connections between the fields of microbiology and medicine.

Critical Thinking

Exercise:

Problem:

Explain how the discovery of fermented foods likely benefited our ancestors.

Exercise:

Problem:

What evidence would you use to support this statement: Ancient people thought that disease was transmitted by things they could not see.

A Systematic Approach

LEARNING OBJECTIVES

- Describe how microorganisms are classified and distinguished as unique species
- Compare historical and current systems of taxonomy used to classify microorganisms

Once microbes became visible to humans with the help of microscopes, scientists began to realize their enormous diversity. Microorganisms vary in all sorts of ways, including their size, their appearance, and their rates of reproduction. To study this incredibly diverse new array of organisms, researchers needed a way to systematically organize them.

The Science of Taxonomy

Taxonomy is the classification, description, identification, and naming of living organisms. Classification is the practice of organizing organisms into different groups based on their shared characteristics. The most famous early taxonomist was a Swedish botanist, zoologist, and physician named Carolus Linnaeus (1701–1778). In 1735, Linnaeus published *Systema Naturae*, an 11-page booklet in which he proposed the Linnaean taxonomy, a system of categorizing and naming organisms using a standard format so scientists could discuss organisms using consistent terminology. He continued to revise and add to the book, which grew into multiple volumes ([\[link\]](#)).



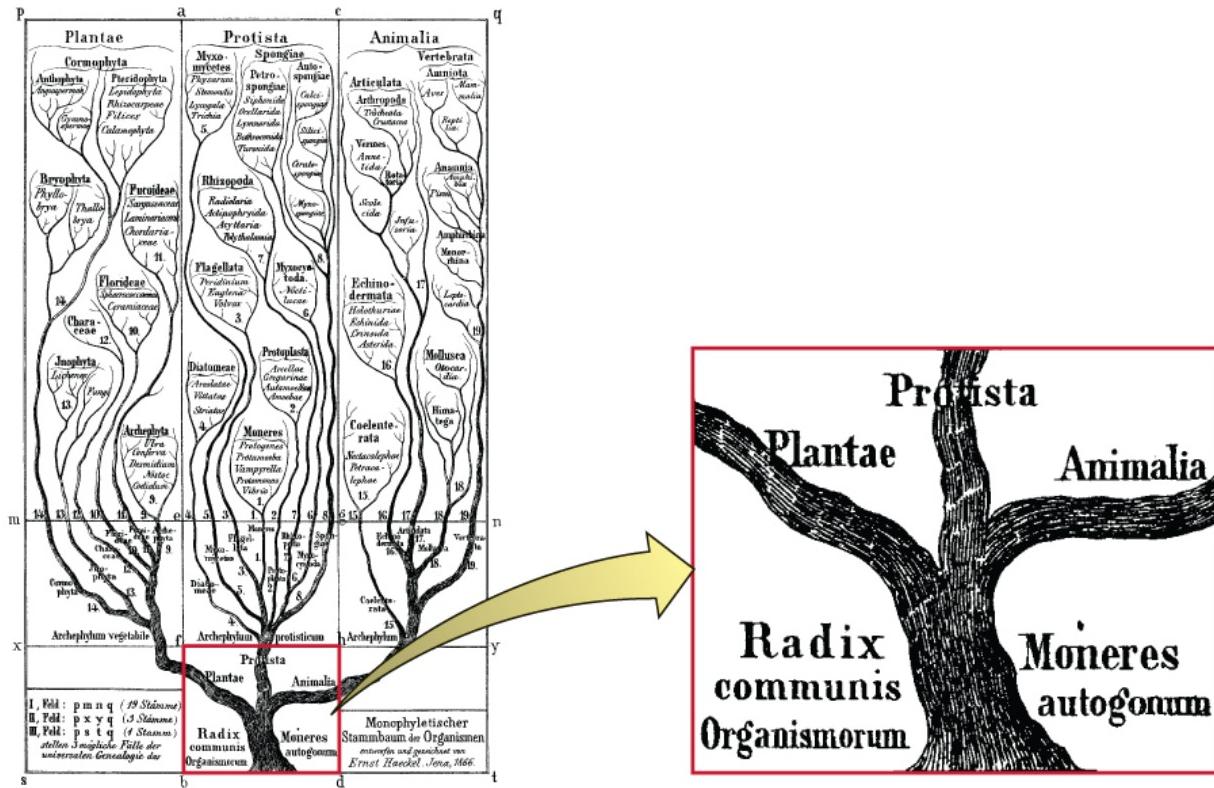
Swedish botanist, zoologist, and physician Carolus Linnaeus developed a new system for categorizing plants and animals. In this 1853 portrait by Hendrik Hollander, Linnaeus is holding a twinflower, named *Linnaea borealis* in his honor.

In his taxonomy, Linnaeus divided the natural world into three kingdoms: animal, plant, and mineral (the mineral kingdom was later abandoned). Within the animal and plant kingdoms, he grouped organisms using a hierarchy of increasingly specific levels and sublevels based on their similarities. The names of the levels in Linnaeus's original taxonomy were kingdom, class, order, family, genus (plural: genera), and species. Species was, and continues to be, the most specific and basic taxonomic unit.

Evolving Trees of Life (Phylogenies)

With advances in technology, other scientists gradually made refinements to the Linnaean system and eventually created new systems for classifying organisms. In the 1800s, there was a growing interest in developing taxonomies that took into account the evolutionary relationships, or **phylogenies**, of all different species of organisms on earth. One way to depict these relationships is via a diagram called a phylogenetic tree (or tree of life). In these diagrams, groups of organisms are arranged by how closely related they are thought to be. In early phylogenetic trees, the relatedness of organisms was inferred by their visible similarities, such as the presence or absence of hair or the number of limbs. Now, the analysis is more complicated. Today, phylogenetic analyses include genetic, biochemical, and embryological comparisons, as will be discussed later in this chapter.

Linnaeus's tree of life contained just two main branches for all living things: the animal and plant kingdoms. In 1866, Ernst Haeckel, a German biologist, philosopher, and physician, proposed another kingdom, Protista, for unicellular organisms ([\[link\]](#)). He later proposed a fourth kingdom, Monera, for unicellular organisms whose cells lack nuclei, like bacteria.

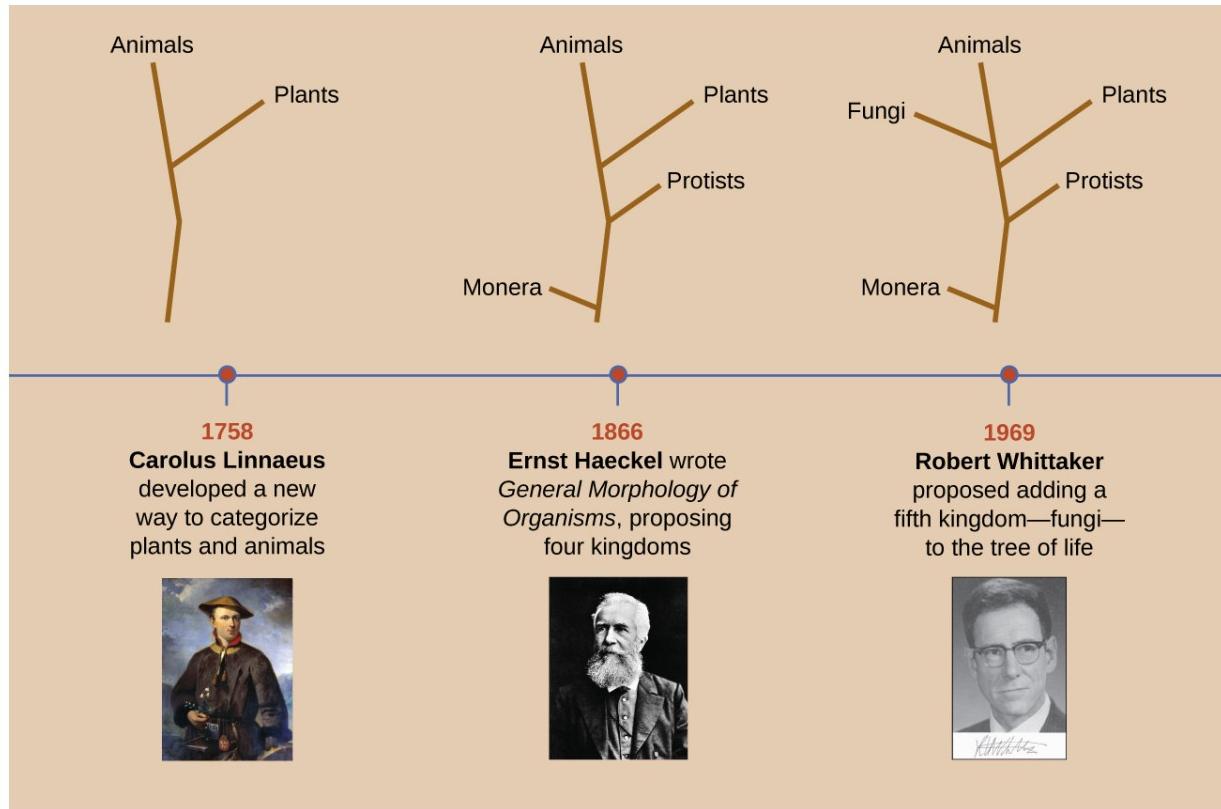


Ernst Haeckel's rendering of the tree of life, from his 1866 book *General Morphology of Organisms*, contained three kingdoms: Plantae, Protista, and Animalia. He later added a fourth kingdom, Monera, for unicellular organisms lacking a nucleus.

Nearly 100 years later, in 1969, American ecologist Robert Whittaker (1920–1980) proposed adding another kingdom—Fungi—in his tree of life. Whittaker's tree also contained a level of categorization above the kingdom level—the empire or superkingdom level—to distinguish between organisms that have membrane-bound nuclei in their cells (**eukaryotes**) and those that do not (**prokaryotes**). Empire Prokaryota contained just the Kingdom Monera. The Empire Eukaryota contained the other four kingdoms: Fungi, Protista, Plantae, and Animalia. Whittaker's five-kingdom tree was considered the standard phylogeny for many years.

[\[link\]](#) shows how the tree of life has changed over time. Note that viruses are not found in any of these trees. That is because they are not made up of

cells and thus it is difficult to determine where they would fit into a tree of life.



This timeline shows how the shape of the tree of life has changed over the centuries. Even today, the taxonomy of living organisms is continually being reevaluated and refined with advances in technology.

Note:

- Briefly summarize how our evolving understanding of microorganisms has contributed to changes in the way that organisms are classified.

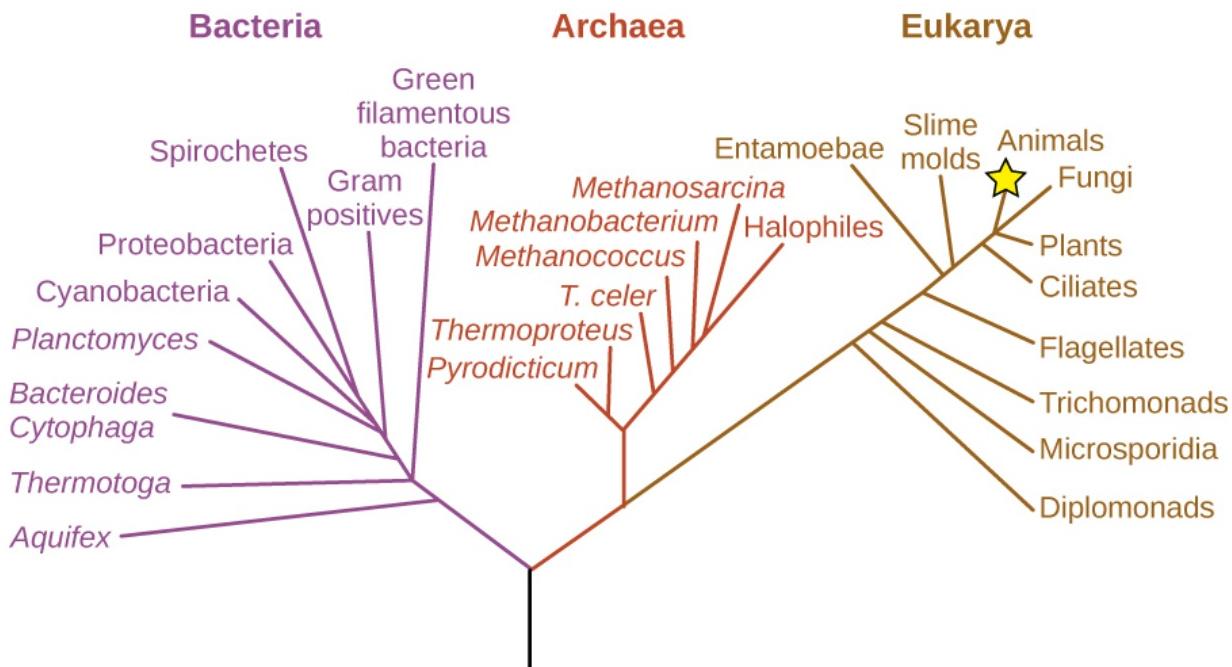
The Role of Genetics in Modern Taxonomy

Haeckel's and Whittaker's trees presented hypotheses about the phylogeny of different organisms based on readily observable characteristics. But the advent of molecular genetics in the late 20th century revealed other ways to organize phylogenetic trees. Genetic methods allow for a standardized way to compare all living organisms without relying on observable characteristics that can often be subjective. Modern taxonomy relies heavily on comparing the nucleic acids (deoxyribonucleic acid [DNA] or ribonucleic acid [RNA]) or proteins from different organisms. The more similar the nucleic acids and proteins are between two organisms, the more closely related they are considered to be.

In the 1970s, American microbiologist Carl Woese discovered what appeared to be a “living record” of the evolution of organisms. He and his collaborator George Fox created a genetics-based tree of life based on similarities and differences they observed in the small subunit ribosomal RNA (rRNA) of different organisms. In the process, they discovered that a certain type of bacteria, called archaeabacteria (now known simply as archaea), were significantly different from other bacteria and eukaryotes in terms of the sequence of small subunit rRNA. To accommodate this difference, they created a tree with three Domains above the level of Kingdom: Archaea, Bacteria, and Eukarya ([\[link\]](#)). Genetic analysis of the small subunit rRNA suggests archaea, bacteria, and eukaryotes all evolved from a common ancestral cell type. The tree is skewed to show a closer evolutionary relationship between Archaea and Eukarya than they have to Bacteria.

Phylogenetic Tree of Life

 = You are here



Woese and Fox's phylogenetic tree contains three domains: Bacteria, Archaea, and Eukarya. Domains Archaea and Bacteria contain all prokaryotic organisms, and Eukarya contains all eukaryotic organisms.
(credit: modification of work by Eric Gaba)

Scientists continue to use analysis of RNA, DNA, and proteins to determine how organisms are related. One interesting, and complicating, discovery is that of horizontal gene transfer—when a gene of one species is absorbed into another organism’s genome. Horizontal gene transfer is especially common in microorganisms and can make it difficult to determine how organisms are evolutionarily related. Consequently, some scientists now think in terms of “webs of life” rather than “trees of life.”

Note:

- In modern taxonomy, how do scientists determine how closely two organisms are related?
- Explain why the branches on the “tree of life” all originate from a single “trunk.”

Naming Microbes

In developing his taxonomy, Linnaeus used a system of **binomial nomenclature**, a two-word naming system for identifying organisms by genus and species. For example, modern humans are in the genus *Homo* and have the species name *sapiens*, so their scientific name in binomial nomenclature is *Homo sapiens*. In binomial nomenclature, the genus part of the name is always capitalized; it is followed by the species name, which is not capitalized. Both names are italicized.

Taxonomic names in the 18th through 20th centuries were typically derived from Latin, since that was the common language used by scientists when taxonomic systems were first created. Today, newly discovered organisms can be given names derived from Latin, Greek, or English. Sometimes these names reflect some distinctive trait of the organism; in other cases, microorganisms are named after the scientists who discovered them. The archaeon *Haloquadratum walsbyi* is an example of both of these naming schemes. The genus, *Haloquadratum*, describes the microorganism’s saltwater habitat (*halo* is derived from the Greek word for “salt”) as well as the arrangement of its square cells, which are arranged in square clusters of four cells (*quadratum* is Latin for “foursquare”). The species, *walsbyi*, is named after Anthony Edward Walsby, the microbiologist who discovered *Haloquadratum walsbyi* in 1980. While it might seem easier to give an organism a common descriptive name—like a red-headed woodpecker—we can imagine how that could become problematic. What happens when another species of woodpecker with red head coloring is discovered? The systematic nomenclature scientists use eliminates this potential problem by assigning each organism a single, unique two-word name that is recognized by scientists all over the world.

In this text, we will typically abbreviate an organism's genus and species after its first mention. The abbreviated form is simply the first initial of the genus, followed by a period and the full name of the species. For example, the bacterium *Escherichia coli* is shortened to *E. coli* in its abbreviated form. You will encounter this same convention in other scientific texts as well.

Bergey's Manuals

Whether in a tree or a web, microbes can be difficult to identify and classify. Without easily observable macroscopic features like feathers, feet, or fur, scientists must capture, grow, and devise ways to study their biochemical properties to differentiate and classify microbes. Despite these hurdles, a group of microbiologists created and updated a set of manuals for identifying and classifying microorganisms. First published in 1923 and since updated many times, *Bergey's Manual of Determinative Bacteriology* and *Bergey's Manual of Systematic Bacteriology* are the standard references for identifying and classifying different prokaryotes. ([Appendix D](#) of this textbook is partly based on Bergey's manuals; it shows how the organisms that appear in this textbook are classified.) Because so many bacteria look identical, methods based on nonvisual characteristics must be used to identify them. For example, biochemical tests can be used to identify chemicals unique to certain species. Likewise, serological tests can be used to identify specific antibodies that will react against the proteins found in certain species. Ultimately, DNA and rRNA sequencing can be used both for identifying a particular bacterial species and for classifying newly discovered species.

Note:

- What is binomial nomenclature and why is it a useful tool for naming organisms?
- Explain why a resource like one of Bergey's manuals would be helpful in identifying a microorganism in a sample.

Note:**Same Name, Different Strain**

Within one species of microorganism, there can be several subtypes called strains. While different strains may be nearly identical genetically, they can have very different attributes. The bacterium *Escherichia coli* is infamous for causing food poisoning and traveler's diarrhea. However, there are actually many different strains of *E. coli*, and they vary in their ability to cause disease.

One pathogenic (disease-causing) *E. coli* strain that you may have heard of is *E. coli* O157:H7. In humans, infection from *E. coli* O157:H7 can cause abdominal cramps and diarrhea. Infection usually originates from contaminated water or food, particularly raw vegetables and undercooked meat. In the 1990s, there were several large outbreaks of *E. coli* O157:H7 thought to have originated in undercooked hamburgers.

While *E. coli* O157:H7 and some other strains have given *E. coli* a bad name, most *E. coli* strains do not cause disease. In fact, some can be helpful. Different strains of *E. coli* found naturally in our gut help us digest our food, provide us with some needed chemicals, and fight against pathogenic microbes.

Note:

Learn more about phylogenetic trees by exploring the Wellcome Trust's interactive Tree of Life. The [website](#) contains information, photos, and

animations about many different organisms. Select two organisms to see how they are evolutionarily related.

- Carolus Linnaeus developed a taxonomic system for categorizing organisms into related groups.
- **Binomial nomenclature** assigns organisms Latinized scientific names with a genus and species designation.
- A **phylogenetic tree** is a way of showing how different organisms are thought to be related to one another from an evolutionary standpoint.
- The first phylogenetic tree contained kingdoms for plants and animals; Ernst Haeckel proposed adding kingdom for protists.
- Robert Whittaker's tree contained five kingdoms: Animalia, Plantae, Protista, Fungi, and Monera.
- Carl Woese used small subunit ribosomal RNA to create a phylogenetic tree that groups organisms into three domains based on their genetic similarity.
- Bergey's manuals of determinative and systemic bacteriology are the standard references for identifying and classifying bacteria, respectively.
- Bacteria can be identified through biochemical tests, DNA/RNA analysis, and serological testing methods.

Critical Thinking

Exercise:

Problem:

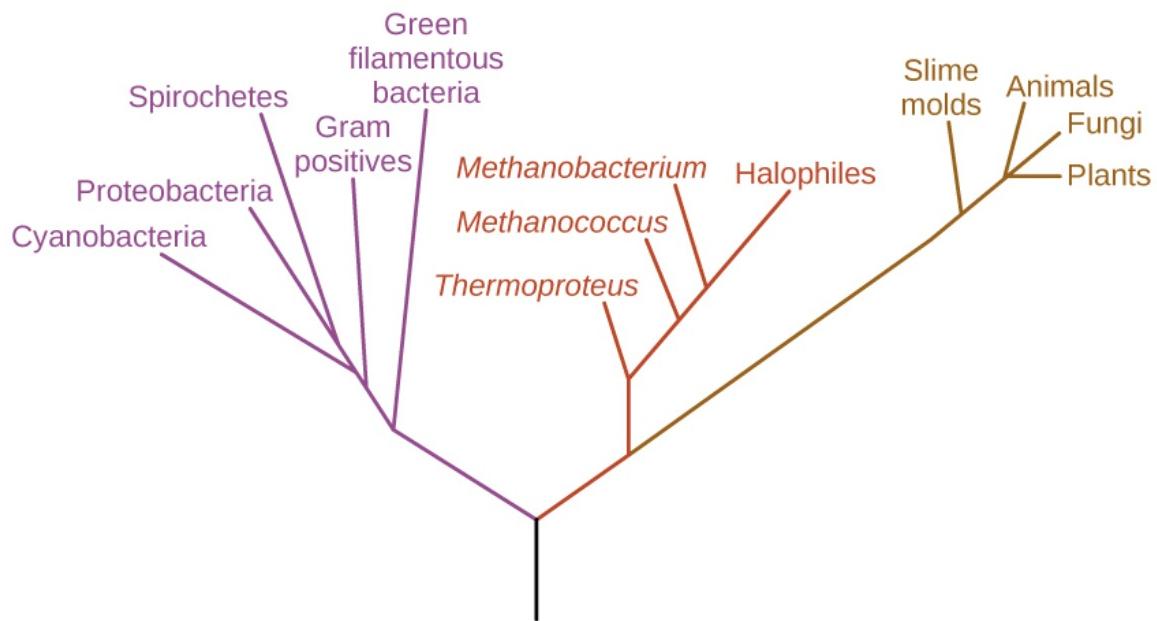
Why is using binomial nomenclature more useful than using common names?

Exercise:

Problem:

Label the three Domains found on modern phylogenetic trees.

Phylogenetic Tree of Life

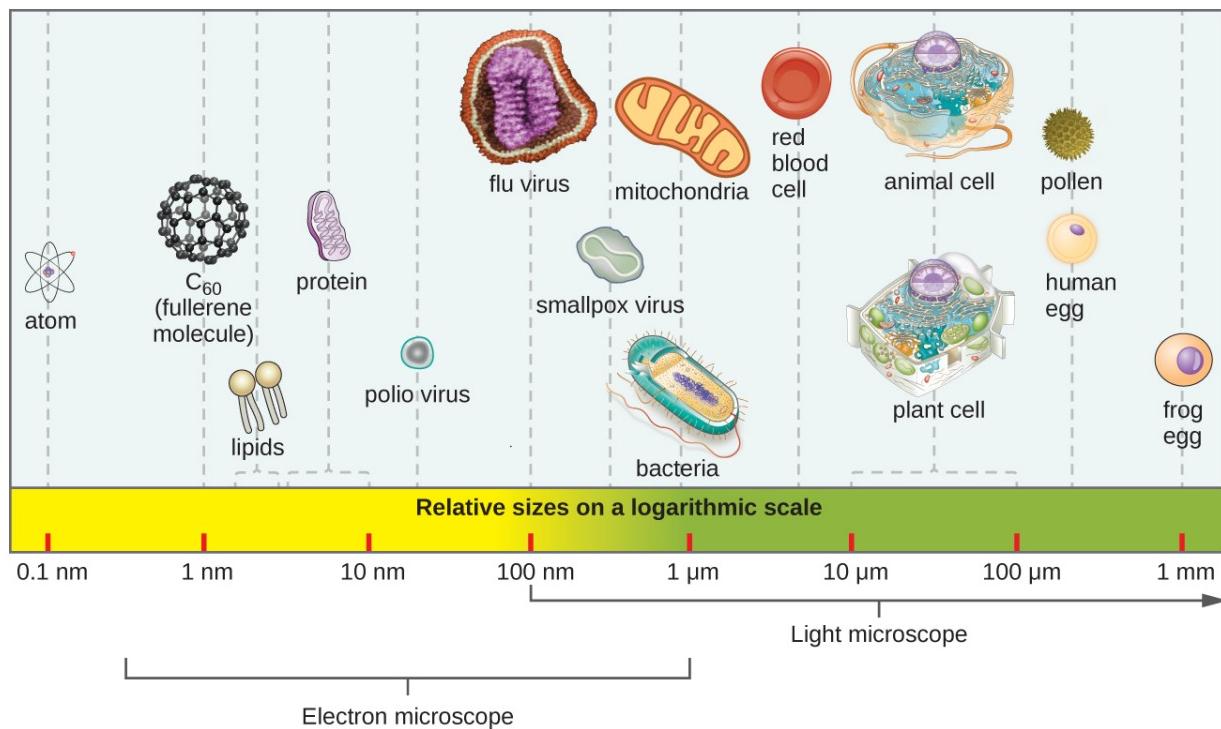


Types of Microorganisms

LEARNING OBJECTIVES

- List the various types of microorganisms and describe their defining characteristics
- Give examples of different types of cellular and viral microorganisms and infectious agents
- Describe the similarities and differences between archaea and bacteria
- Provide an overview of the field of microbiology

Most microbes are unicellular and small enough that they require artificial magnification to be seen. However, there are some unicellular microbes that are visible to the naked eye, and some multicellular organisms that are microscopic. An object must measure about 100 micrometers (μm) to be visible without a microscope, but most microorganisms are many times smaller than that. For some perspective, consider that a typical animal cell measures roughly 10 μm across but is still microscopic. Bacterial cells are typically about 1 μm , and viruses can be 10 times smaller than bacteria ([\[link\]](#)). See [\[link\]](#) for units of length used in microbiology.



The relative sizes of various microscopic and nonmicroscopic objects. Note that a typical virus measures about 100 nm, 10 times smaller than a typical bacterium (~1 μm), which is at least 10 times smaller than a typical plant or animal cell (~10–100 μm). An object must measure about 100 μm to be visible without a microscope.

Units of Length Commonly Used in Microbiology

Metric Unit	Meaning of Prefix	Metric Equivalent
meter (m)	—	$1 \text{ m} = 10^0 \text{ m}$

Units of Length Commonly Used in Microbiology

Metric Unit	Meaning of Prefix	Metric Equivalent
decimeter (dm)	1/10	$1 \text{ dm} = 0.1 \text{ m} = 10^{-1} \text{ m}$
centimeter (cm)	1/100	$1 \text{ cm} = 0.01 \text{ m} = 10^{-2} \text{ m}$
millimeter (mm)	1/1000	$1 \text{ mm} = 0.001 \text{ m} = 10^{-3} \text{ m}$
micrometer (μm)	1/1,000,000	$1 \mu\text{m} = 0.000001 \text{ m} = 10^{-6} \text{ m}$
nanometer (nm)	1/1,000,000,000	$1 \text{ nm} = 0.000000001 \text{ m} = 10^{-9} \text{ m}$

Microorganisms differ from each other not only in size, but also in structure, habitat, metabolism, and many other characteristics. While we typically think of microorganisms as being unicellular, there are also many multicellular organisms that are too small to be seen without a microscope. Some microbes, such as viruses, are even **acellular** (not composed of cells).

Microorganisms are found in each of the three domains of life: Archaea, Bacteria, and Eukarya. Microbes within the domains Bacteria and Archaea are all prokaryotes (their cells lack a nucleus), whereas microbes in the domain Eukarya are eukaryotes (their cells have a nucleus). Some microorganisms, such as viruses, do not fall within any of the three domains of life. In this section, we will briefly introduce each of the broad groups of microbes. Later chapters will go into greater depth about the diverse species within each group.

Note:

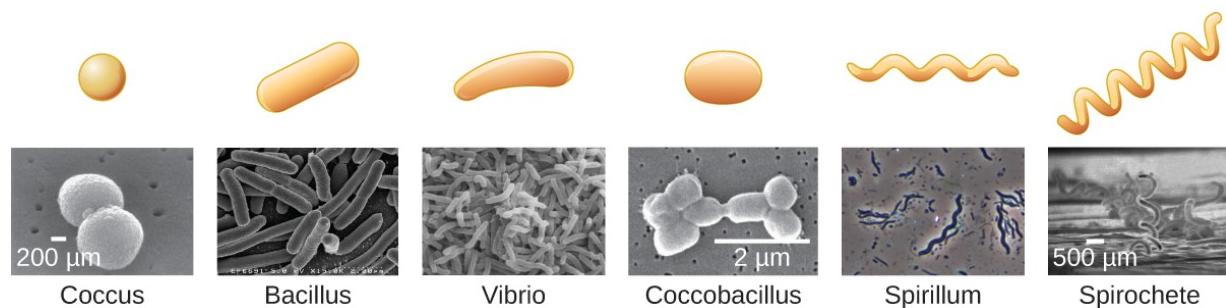


How big is a bacterium or a virus compared to other objects? Check out this [interactive website](#) to get a feel for the scale of different microorganisms.

Prokaryotic Microorganisms

Bacteria are found in nearly every habitat on earth, including within and on humans. Most bacteria are **harmless** or **helpful**, but some are **pathogens**, causing disease in humans and other animals. Bacteria are prokaryotic because their genetic material (DNA) is not housed within a true nucleus. Most bacteria have cell walls that contain peptidoglycan.

Bacteria are often described in terms of their general shape. Common shapes include spherical (coccus), rod-shaped (bacillus), or curved (spirillum, spirochete, or vibrio). [\[link\]](#) shows examples of these shapes.



Common bacterial shapes. Note how coccobacillus is a combination of

spherical (coccus) and rod-shaped (bacillus). (credit “Coccus”: modification of work by Janice Haney Carr, Centers for Disease Control and Prevention; credit “Coccobacillus”: modification of work by Janice Carr, Centers for Disease Control and Prevention; credit “Spirochete”: Centers for Disease Control and Prevention)

They have a wide range of metabolic capabilities and can grow in a variety of environments, using different combinations of nutrients. Some bacteria are photosynthetic, such as oxygenic cyanobacteria and anoxygenic green sulfur and green nonsulfur bacteria; these bacteria use energy derived from sunlight, and fix carbon dioxide for growth. Other types of bacteria are nonphotosynthetic, obtaining their energy from organic or inorganic compounds in their environment.

Archaea are also unicellular prokaryotic organisms. Archaea and bacteria have different evolutionary histories, as well as significant differences in genetics, metabolic pathways, and the composition of their cell walls and membranes. Unlike most bacteria, archaeal cell walls do not contain peptidoglycan, but their cell walls are often composed of a similar substance called pseudopeptidoglycan. Like bacteria, archaea are found in nearly every habitat on earth, even extreme environments that are very cold, very hot, very basic, or very acidic ([\[link\]](#)). Some archaea live in the human body, but none have been shown to be human pathogens.



Some archaea live in extreme environments, such as the Morning Glory pool, a hot spring in Yellowstone National Park. The color differences in the pool result from the different communities of microbes that are able to thrive at various water temperatures.

Note:

- What are the two main types of prokaryotic organisms?
- Name some of the defining characteristics of each type.

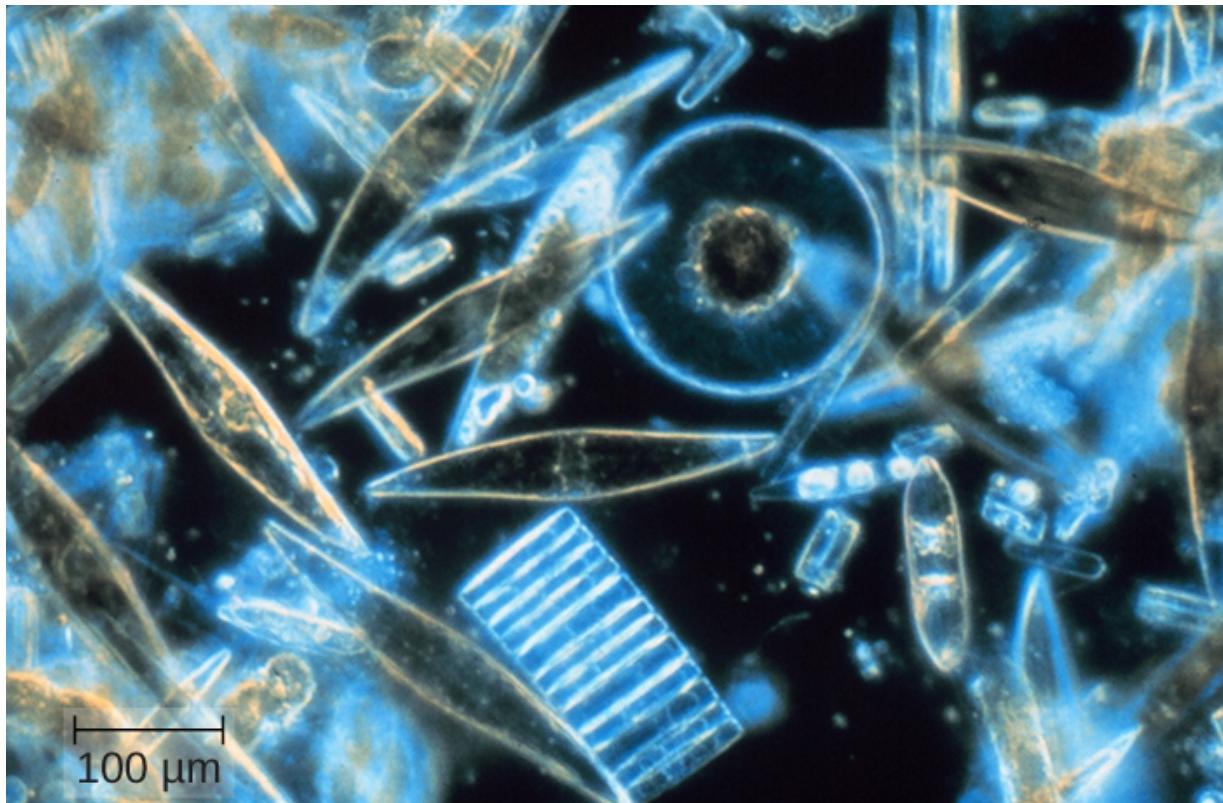
Eukaryotic Microorganisms

The domain Eukarya contains all eukaryotes, including uni- or multicellular eukaryotes such as protists, fungi, plants, and animals. The major defining characteristic of eukaryotes is that their cells contain a nucleus.

Protists

Protists are unicellular eukaryotes that are not plants, animals, or fungi. Algae and protozoa are examples of protists.

Algae (singular: alga) are plant-like protists that can be either unicellular or multicellular ([\[link\]](#)). Their cells are surrounded by cell walls made of cellulose, a type of carbohydrate. Algae are photosynthetic organisms that extract energy from the sun and release oxygen and carbohydrates into their environment. Because other organisms can use their waste products for energy, algae are important parts of many ecosystems. Many consumer products contain ingredients derived from algae, such as carrageenan or alginic acid, which are found in some brands of ice cream, salad dressing, beverages, lipstick, and toothpaste. A derivative of algae also plays a prominent role in the microbiology laboratory. Agar, a gel derived from algae, can be mixed with various nutrients and used to grow microorganisms in a Petri dish. Algae are also being developed as a possible source for biofuels.



Assorted diatoms, a kind of algae, live in annual sea ice in McMurdo Sound, Antarctica. Diatoms range in size from 2 μm to 200 μm and are visualized here using light microscopy. (credit: modification of work by National Oceanic and Atmospheric Administration)

Protozoa (singular: protozoan) are protists that make up the backbone of many food webs by providing nutrients for other organisms. Protozoa are very diverse. Some protozoa move with help from hair-like structures called cilia or whip-like structures called flagella. Others extend part of their cell membrane and cytoplasm to propel themselves forward. These cytoplasmic extensions are called pseudopods (“false feet”). Some protozoa are photosynthetic; others feed on organic material. Some are free-living, whereas others are parasitic, only able to survive by extracting nutrients from a host organism. Most protozoa are harmless, but some are pathogens that can cause disease in animals or humans ([\[link\]](#)).



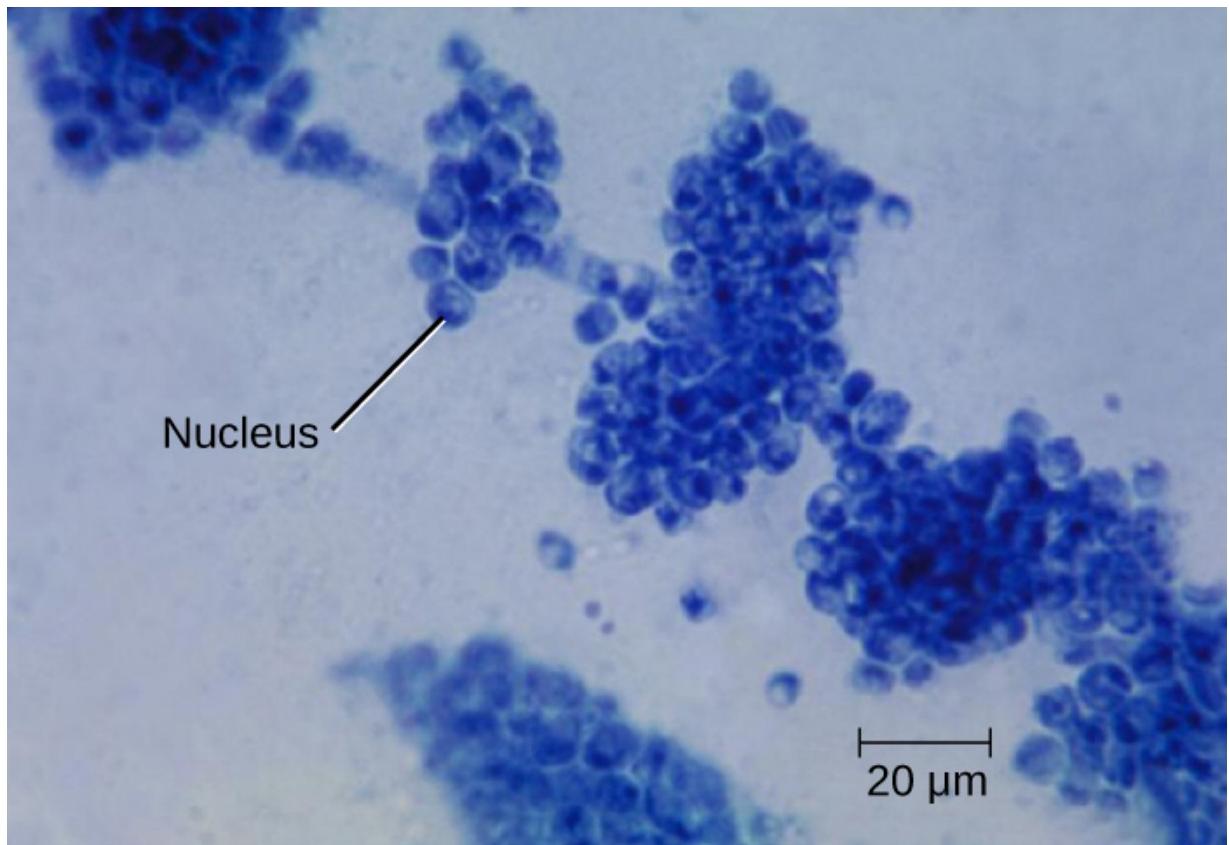
Giardia lamblia, an intestinal protozoan parasite that infects humans and other mammals, causing severe diarrhea. (credit: modification of work by Centers for Disease Control and Prevention)

Fungi

Fungi (singular: fungus) are also eukaryotes. Some multicellular fungi, such as mushrooms, resemble plants, but they are actually quite different.

Fungi are not photosynthetic, and their cell walls are usually made out of chitin rather than cellulose.

Unicellular fungi—yeasts—are included within the study of microbiology. There are more than 1000 known species. Yeasts are found in many different environments, from the deep sea to the human navel. Some yeasts have beneficial uses, such as causing bread to rise and beverages to ferment; but yeasts can also cause food to spoil. Some even cause diseases, such as vaginal yeast infections and oral thrush ([\[link\]](#)).



Candida albicans is a unicellular fungus, or yeast. It is the causative agent of vaginal yeast infections as well as oral thrush, a yeast infection of the mouth that commonly afflicts infants. *C. albicans* has a morphology similar to that of coccus bacteria; however, yeast is a eukaryotic organism (note the nuclei) and is much larger. (credit: modification of work by Centers for Disease Control and Prevention)

Other fungi of interest to microbiologists are multicellular organisms called **molds**. Molds are made up of long filaments that form visible colonies ([\[link\]](#)). Molds are found in many different environments, from soil to rotting food to dank bathroom corners. Molds play a critical role in the decomposition of dead plants and animals. Some molds can cause allergies, and others produce disease-causing metabolites called mycotoxins. Molds have been used to make pharmaceuticals, including penicillin, which is one of the most commonly prescribed antibiotics, and cyclosporine, used to prevent organ rejection following a transplant.



Large colonies of microscopic fungi can often be observed with the naked eye, as seen on the surface of these moldy oranges.

Note:

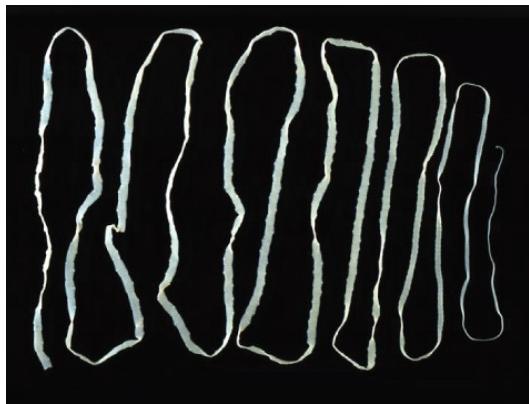
- Name two types of protists and two types of fungi.
- Name some of the defining characteristics of each type.

Helminths

Multicellular parasitic worms called **helminths** are not technically microorganisms, as most are large enough to see without a microscope. However, these worms fall within the field of microbiology because diseases caused by helminths involve microscopic eggs and larvae. One example of a helminth is the guinea worm, or *Dracunculus medinensis*, which causes dizziness, vomiting, diarrhea, and painful ulcers on the legs and feet when the worm works its way out of the skin ([\[link\]](#)). Infection typically occurs after a person drinks water containing water fleas infected by guinea-worm larvae. In the mid-1980s, there were an estimated 3.5 million cases of guinea-worm disease, but the disease has been largely eradicated. In 2014, there were only 126 cases reported, thanks to the coordinated efforts of the World Health Organization (WHO) and other groups committed to improvements in drinking water sanitation.[\[footnote\]](#) [\[footnote\]](#)

C. Greenaway “Dracunculiasis (Guinea Worm Disease).” *Canadian Medical Association Journal* 170 no. 4 (2004):495–500.

World Health Organization. “Dracunculiasis (Guinea-Worm Disease).” WHO. 2015. <http://www.who.int/mediacentre/factsheets/fs359/en/>. Accessed October 2, 2015.



(a)



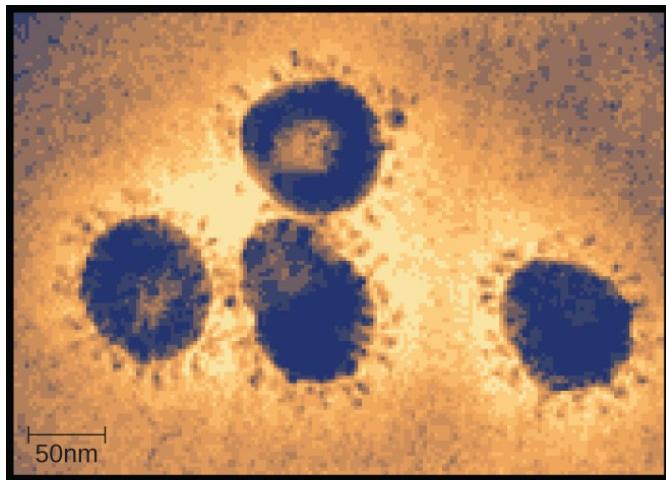
(b)

(a) The beef tapeworm, *Taenia saginata*, infects both cattle and humans. *T. saginata* eggs are microscopic (around 50 µm), but adult worms like the one shown here can reach 4–10 m, taking up residence in the digestive system. (b) An adult guinea worm, *Dracunculus medinensis*, is removed through a lesion in the patient’s skin by winding it around a matchstick. (credit a, b: modification of work by Centers for Disease Control and Prevention)

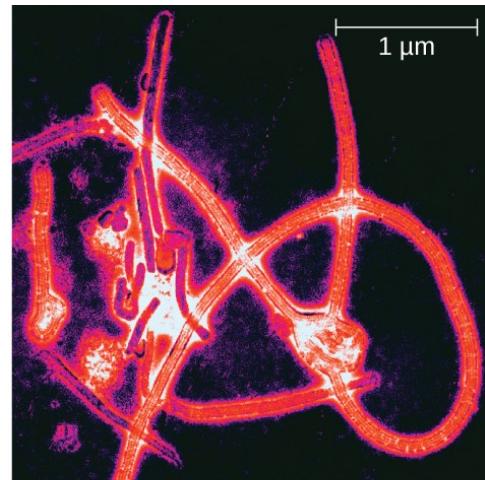
Viruses

Viruses are **acellular** microorganisms, which means they are not composed of cells. Essentially, a virus consists of proteins and genetic material—either DNA or RNA, but never both—that are inert outside of a host organism. However, by incorporating themselves into a host cell, viruses are able to co-opt the host’s cellular mechanisms to multiply and infect other hosts.

Viruses can infect all types of cells, from human cells to the cells of other microorganisms. In humans, viruses are responsible for numerous diseases, from the common cold to deadly Ebola ([\[link\]](#)). However, many viruses do not cause disease. Viruses that infect bacteria have been used to treat pathogenic bacterial infection, a method called Phage Therapy. Some virus are also on the spot as promising therapies to treat certain types of cancers. We will talk more about this when we discuss viral replication.



(a)



(b)

(a) Members of the Coronavirus family can cause respiratory infections like the common cold, severe acute respiratory syndrome (SARS), and Middle East respiratory syndrome (MERS). Here they are viewed under a transmission electron microscope (TEM). (b) Ebolavirus, a member of the Filovirus family, as visualized using a TEM. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by Thomas W. Geisbert)

Note:

- Are helminths microorganisms? Explain why or why not.
- How are viruses different from other microorganisms?

- Microorganisms are very diverse and are found in all three domains of life: Archaea, Bacteria, and Eukarya.
- **Archaea** and **bacteria** are classified as prokaryotes because they lack a cellular nucleus. Archaea differ from bacteria in evolutionary history,

genetics, metabolic pathways, and cell wall and membrane composition.

- Archaea inhabit nearly every environment on earth, but no archaea have been identified as human pathogens.
- **Eukaryotes** studied in microbiology include algae, protozoa, fungi, and helminths.
- **Algae** are plant-like organisms that can be either unicellular or multicellular, and derive energy via photosynthesis.
- **Protozoa** are unicellular organisms with complex cell structures; most are motile.
- Microscopic **fungi** include **molds** and **yeasts**.
- **Helminths** are multicellular parasitic worms. They are included in the field of microbiology because their eggs and larvae are often microscopic.
- **Viruses** are acellular microorganisms that require a host to reproduce.
- The field of microbiology is extremely broad. Microbiologists typically specialize in one of many subfields, but all health professionals need a solid foundation in clinical microbiology.

Critical Thinking

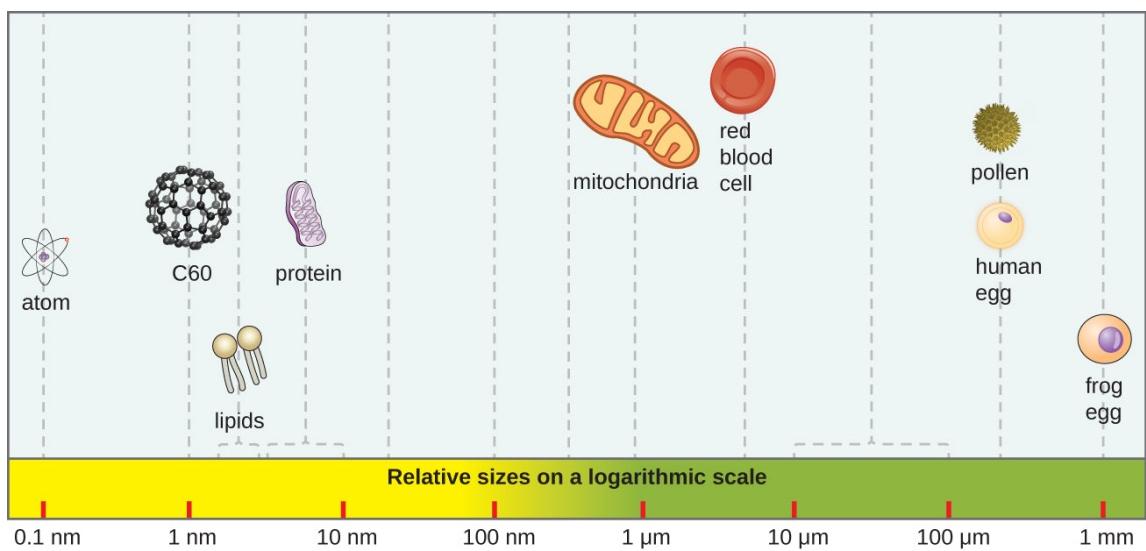
Exercise:

Problem: Contrast the behavior of a virus outside versus inside a cell.

Exercise:

Problem:

Where would a virus, bacterium, animal cell, and a prion belong on this chart?



Observing the microbial world - Introduction

class="introduction"

Different types of microscopy are used to visualize different structures. Brightfield microscopy (left) renders a darker image on a lighter background, producing a clear image of these *Bacillus anthracis* cells in cerebrospinal fluid (the rod-shaped bacterial cells are surrounded by larger white blood cells).

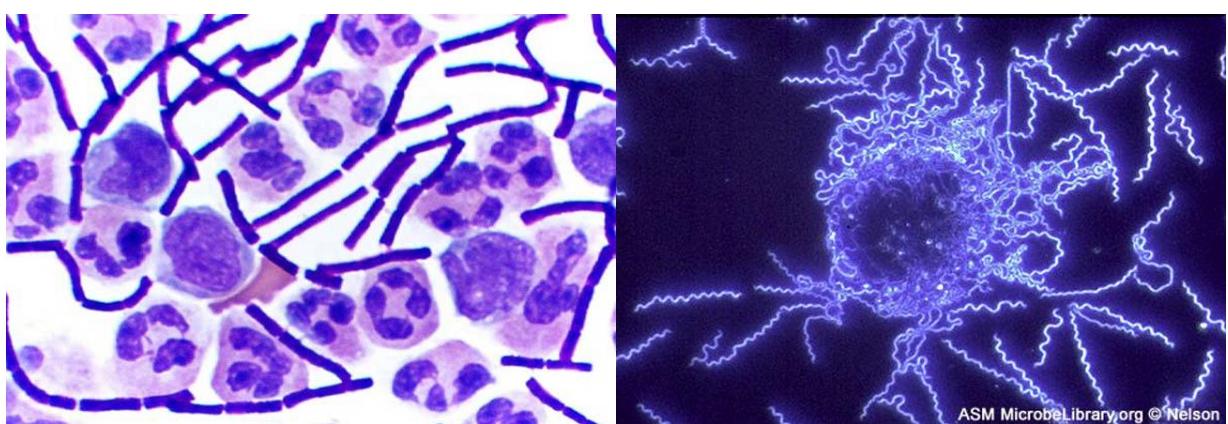
Darkfield microscopy (right) increases contrast, rendering a brighter

image on a
darker
background,
as

demonstrated
by this image
of the
bacterium

Borrelia
burgdorferi,
which causes
Lyme disease.

(credit left:
modification
of work by
Centers for
Disease
Control and
Prevention;
credit right:
modification
of work by
American
Society for
Microbiology
)



When we look at a rainbow, its colors span the full spectrum of light that the human eye can detect and differentiate. Each hue represents a different frequency of visible light, processed by our eyes and brains and rendered as red, orange, yellow, green, or one of the many other familiar colors that have always been a part of the human experience. But only recently have humans developed an understanding of the properties of light that allow us to see images in color.

Over the past several centuries, we have learned to manipulate light to peer into previously invisible worlds—those too small or too far away to be seen by the naked eye. Through a microscope, we can examine microbial cells and colonies, using various techniques to manipulate color, size, and contrast in ways that help us identify species and diagnose disease.

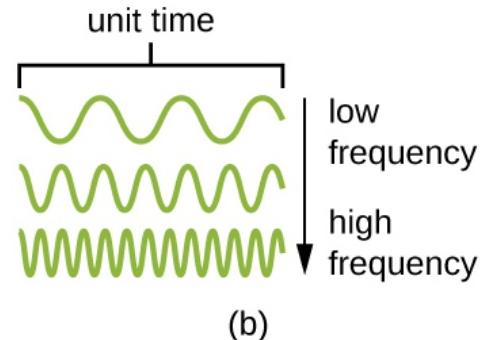
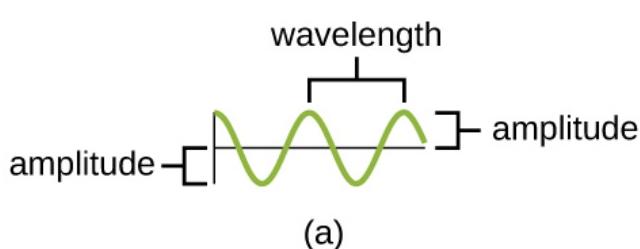
[[link](#)] illustrates how we can apply the properties of light to visualize and magnify images; but these stunning micrographs are just two examples of the numerous types of images we are now able to produce with different microscopic technologies. This chapter explores how various types of microscopes manipulate light in order to provide a window into the world of microorganisms. By understanding how various kinds of microscopes work, we can produce highly detailed images of microbes that can be useful for both research and clinical applications.

The Properties of Light

LEARNING OBJECTIVES

- Identify and define the characteristics of electromagnetic radiation (EMR) used in microscopy
- Explain how lenses are used in microscopy to manipulate visible and ultraviolet (UV) light
- Define refraction, magnification, resolution, and contrast

Visible light consists of electromagnetic waves that behave like other waves. Hence, many of the properties of light that are relevant to microscopy can be understood in terms of light's behavior as a wave. An important property of light waves is the **wavelength**, or the distance between one peak of a wave and the next peak. The height of each peak (or depth of each trough) is called the **amplitude**. In contrast, the **frequency** of the wave is the rate of vibration of the wave, or the number of wavelengths within a specified time period ([\[link\]](#)).

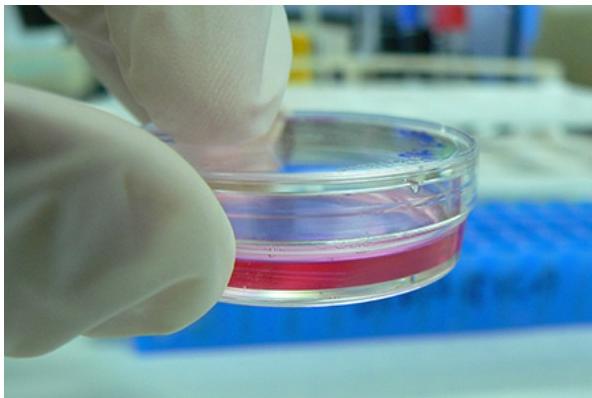


- (a) The amplitude is the height of a wave, whereas the wavelength is the distance between one peak and the next. (b) These waves have different frequencies, or rates of vibration. The wave at the top has the

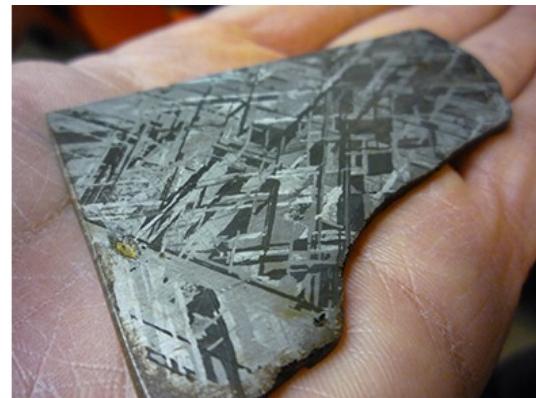
lowest frequency, since it has the fewest peaks per unit time. The wave at the bottom has the highest frequency.

Interactions of Light

Light waves interact with materials by being reflected, absorbed, or transmitted. **Reflection** occurs when a wave bounces off of a material. For example, a red piece of cloth may reflect red light to our eyes while absorbing other colors of light. **Absorbance** occurs when a material captures the energy of a light wave. In the case of glow-in-the-dark plastics, the energy from light can be absorbed and then later re-emitted as another form of phosphorescence. Transmission occurs when a wave travels through a material, like light through glass (the process of transmission is called **transmittance**). When a material allows a large proportion of light to be transmitted, it may do so because it is thinner, or more transparent (having more **transparency** and less **opacity**). [\[link\]](#) illustrates the difference between transparency and opacity.



(a)



(b)

- (a) A Petri dish is made of transparent plastic or glass, which allows transmission of a high proportion of light. This transparency allows us to see through the sides of the dish to view the contents. (b) This slice of an iron meteorite is opaque (i.e., it has opacity). Light is not

transmitted through the material, making it impossible to see the part of the hand covered by the object. (credit a: modification of work by Umberto Salvagnin; credit b: modification of work by “Waifer X”/Flickr)

Light waves can also interact with each other by **interference**, creating complex patterns of motion. Dropping two pebbles into a puddle causes the waves on the puddle’s surface to interact, creating complex interference patterns. Light waves can interact in the same way.

In addition to interfering with each other, light waves can also interact with small objects or openings by bending or scattering. This is called **diffraction**. Diffraction is larger when the object is smaller relative to the wavelength of the light (the distance between two consecutive peaks of a light wave). Often, when waves diffract in different directions around an obstacle or opening, they will interfere with each other.

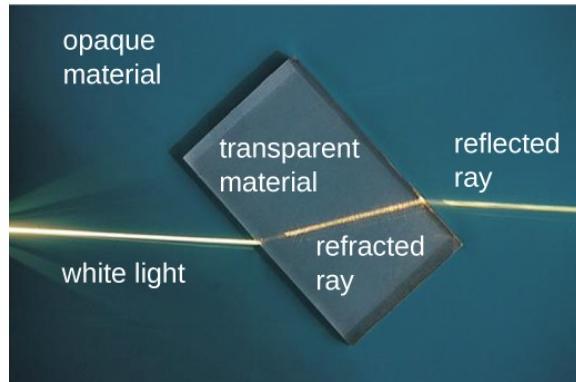
Note:

- If a light wave has a long wavelength, is it likely to have a low or high frequency?
- If an object is transparent, does it reflect, absorb, or transmit light?

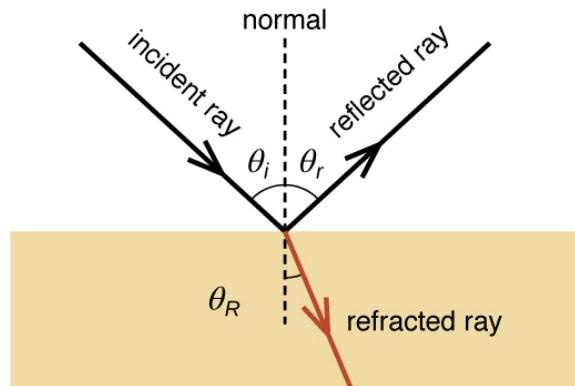
Lenses and Refraction

In the context of microscopy, **refraction** is perhaps the most important behavior exhibited by light waves. Refraction occurs when light waves change direction as they enter a new medium ([\[link\]](#)). Different transparent materials transmit light at different speeds; thus, light can change speed when passing from one material to another. This change in speed usually

also causes a change in direction (refraction), with the degree of change dependent on the angle of the incoming light.



(a)



(b)

(a) Refraction occurs when light passes from one medium, such as air, to another, such as glass, changing the direction of the light rays. (b)

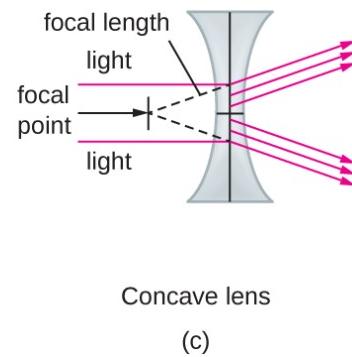
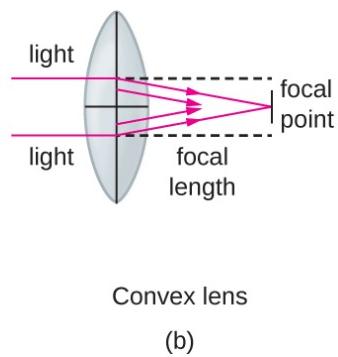
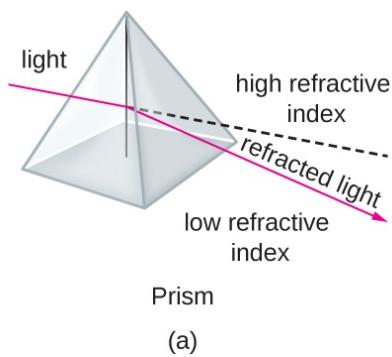
As shown in this diagram, light rays passing from one medium to another may be either refracted or reflected.

The extent to which a material slows transmission speed relative to empty space is called the **refractive index** of that material. Large differences between the refractive indices of two materials will result in a large amount of refraction when light passes from one material to the other. For example, light moves much more slowly through water than through air, so light entering water from air can change direction greatly. We say that the water has a higher refractive index than air ([\[link\]](#)).



This straight pole appears to bend at an angle as it enters the water. This optical illusion is due to the large difference between the refractive indices of air and water.

When light crosses a boundary into a material with a higher refractive index, its direction turns to be closer to perpendicular to the boundary (i.e., more toward a normal to that boundary; see [\[link\]](#)). This is the principle behind lenses. We can think of a lens as an object with a curved boundary (or a collection of prisms) that collects all of the light that strikes it and refracts it so that it all meets at a single point called the **image point** (**focus**). A convex lens can be used to magnify because it can focus at closer range than the human eye, producing a larger image. Concave lenses and mirrors can also be used in microscopes to redirect the light path. [\[link\]](#) shows the **focal point** (the image point when light entering the lens is parallel) and the **focal length** (the distance to the focal point) for convex and concave lenses.



- (a) A lens is like a collection of prisms, such as the one shown here.
- (b) When light passes through a convex lens, it is refracted toward a focal point on the other side of the lens. The focal length is the distance to the focal point. (c) Light passing through a concave lens is refracted away from a focal point in front of the lens.

The human eye contains a lens that enables us to see images. This lens focuses the light reflecting off of objects in front of the eye onto the surface of the retina, which is like a screen in the back of the eye. Artificial lenses placed in front of the eye (contact lenses, glasses, or microscopic lenses) focus light before it is focused (again) by the lens of the eye, manipulating the image that ends up on the retina (e.g., by making it appear larger).

Images are commonly manipulated by controlling the distances between the object, the lens, and the screen, as well as the curvature of the lens. For example, for a given amount of curvature, when an object is closer to the lens, the focal points are farther from the lens. As a result, it is often necessary to manipulate these distances to create a focused image on a screen. Similarly, more curvature creates image points closer to the lens and a larger image when the image is in focus. This property is often described in terms of the focal distance, or distance to the focal point.

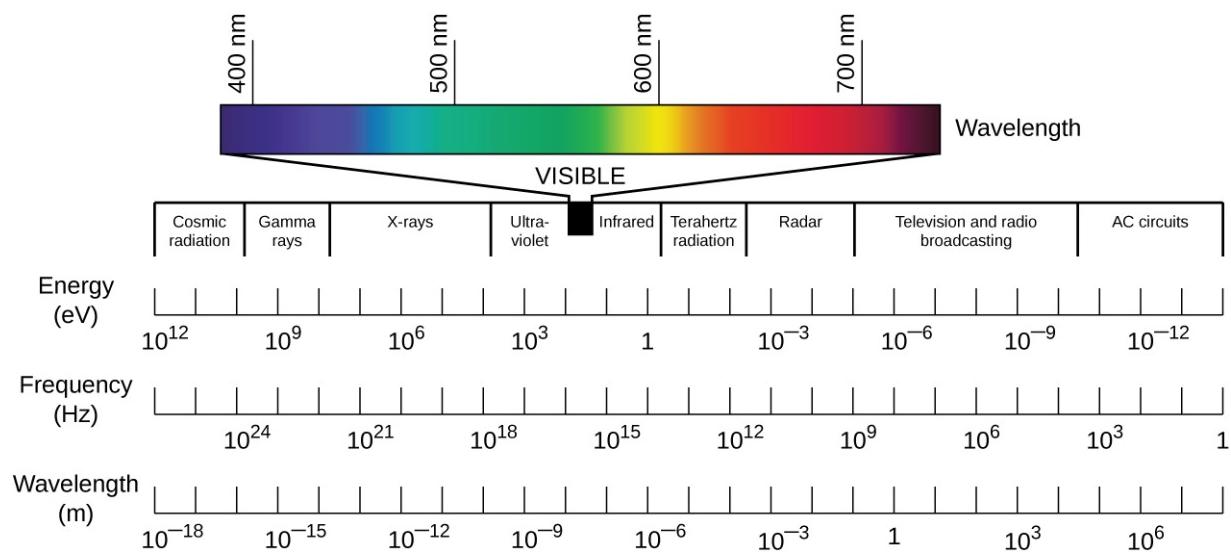
Note:

- Explain how a lens focuses light at the image point.

- Name some factors that affect the focal length of a lens.

Electromagnetic Spectrum and Color

Visible light is just one form of electromagnetic radiation (EMR), a type of energy that is all around us. Other forms of EMR include microwaves, X-rays, and radio waves, among others. The different types of EMR fall on the electromagnetic spectrum, which is defined in terms of wavelength and frequency. The spectrum of visible light occupies a relatively small range of frequencies between infrared and ultraviolet light ([\[link\]](#)).

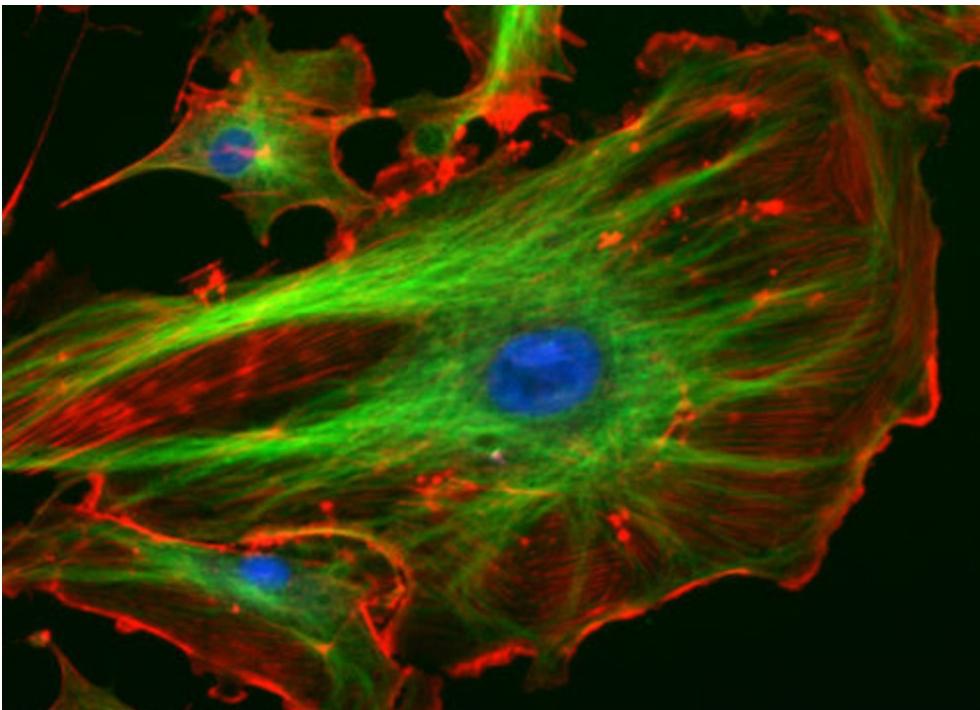


The electromagnetic spectrum ranges from high-frequency gamma rays to low-frequency radio waves. Visible light is the relatively small range of electromagnetic frequencies that can be sensed by the human eye. On the electromagnetic spectrum, visible light falls between ultraviolet and infrared light. (credit: modification of work by Johannes Ahlmann)

Whereas wavelength represents the distance between adjacent peaks of a light wave, frequency, in a simplified definition, represents the rate of oscillation. Waves with higher frequencies have shorter wavelengths and, therefore, have more oscillations per unit time than lower-frequency waves. Higher-frequency waves also contain more energy than lower-frequency waves. This energy is delivered as elementary particles called photons. Higher-frequency waves deliver more energetic photons than lower-frequency waves.

Photons with different energies interact differently with the retina. In the spectrum of visible light, each color corresponds to a particular frequency and wavelength ([\[link\]](#)). The lowest frequency of visible light appears as the color red, whereas the highest appears as the color violet. When the retina receives visible light of many different frequencies, we perceive this as white light. However, white light can be separated into its component colors using refraction. If we pass white light through a prism, different colors will be refracted in different directions, creating a rainbow-like spectrum on a screen behind the prism. This separation of colors is called **dispersion**, and it occurs because, for a given material, the refractive index is different for different frequencies of light.

Certain materials can refract nonvisible forms of EMR and, in effect, transform them into visible light. Certain **fluorescent** dyes, for instance, absorb ultraviolet or blue light and then use the energy to emit photons of a different color, giving off light rather than simply vibrating. This occurs because the energy absorption causes electrons to jump to higher energy states, after which they then almost immediately fall back down to their ground states, emitting specific amounts of energy as photons. Not all of the energy is emitted in a given photon, so the emitted photons will be of lower energy and, thus, of lower frequency than the absorbed ones. Thus, a dye such as Texas red may be excited by blue light, but emit red light; or a dye such as fluorescein isothiocyanate (FITC) may absorb (invisible) high-energy ultraviolet light and emit green light ([\[link\]](#)). In some materials, the photons may be emitted following a delay after absorption; in this case, the process is called **phosphorescence**. Glow-in-the-dark plastic works by using phosphorescent material.



The fluorescent dyes absorbed by these bovine pulmonary artery endothelial cells emit brilliant colors when excited by ultraviolet light under a fluorescence microscope. Various cell structures absorb different dyes. The nuclei are stained blue with 4',6-diamidino-2-phenylindole (DAPI); microtubules are marked green by an antibody bound to FITC; and actin filaments are labeled red with phalloidin bound to tetramethylrhodamine (TRITC). (credit: National Institutes of Health)

Note:

- Which has a higher frequency: red light or green light?
- Explain why dispersion occurs when white light passes through a prism.

- Why do fluorescent dyes emit a different color of light than they absorb?

Magnification, Resolution, and Contrast

Microscopes magnify images and use the properties of light to create useful images of small objects. **Magnification** is defined as the ability of a lens to enlarge the image of an object when compared to the real object. For example, a magnification of $10\times$ means that the image appears 10 times the size of the object as viewed with the naked eye.

Greater magnification typically improves our ability to see details of small objects, but magnification alone is not sufficient to make the most useful images. It is often useful to enhance the **resolution** of objects: the ability to tell that two separate points or objects are separate. A low-resolution image appears fuzzy, whereas a high-resolution image appears sharp. Two factors affect resolution. The first is wavelength. Shorter wavelengths are able to resolve smaller objects; thus, an electron microscope has a much higher resolution than a light microscope, since it uses an electron beam with a very short wavelength, as opposed to the long-wavelength visible light used by a light microscope. The second factor that affects resolution is **numerical aperture**, which is a measure of a lens's ability to gather light. The higher the numerical aperture, the better the resolution.

Note:



Read this [article](#) to learn more about factors that can increase or decrease the numerical aperture of a lens.

Even when a microscope has high resolution, it can be difficult to distinguish small structures in many specimens because microorganisms are relatively transparent. It is often necessary to increase **contrast** to detect different structures in a specimen. Various types of microscopes use different features of light or electrons to increase contrast—visible differences between the parts of a specimen (see [Instruments of Microscopy](#)). Additionally, dyes that bind to some structures but not others can be used to improve the contrast between images of relatively transparent objects (see [Staining Microscopic Specimens](#)).

Note:

- Explain the difference between magnification and resolution.
- Explain the difference between resolution and contrast.
- Name two factors that affect resolution.

Key Concepts and Summary

- Light waves interacting with materials may be **reflected**, **absorbed**, or **transmitted**, depending on the properties of the material.
- Light waves can interact with each other (**interference**) or be distorted by interactions with small objects or openings (**diffraction**).
- **Refraction** occurs when light waves change speed and direction as they pass from one medium to another. Differences in the **refraction indices** of two materials determine the magnitude of directional changes when light passes from one to the other.
- A **lens** is a medium with a curved surface that refracts and focuses light to produce an image.

- Visible light is part of the **electromagnetic spectrum**; light waves of different frequencies and wavelengths are distinguished as colors by the human eye.
- A prism can separate the colors of white light (**dispersion**) because different frequencies of light have different refractive indices for a given material.
- **Fluorescent dyes and phosphorescent** materials can effectively transform nonvisible electromagnetic radiation into visible light.
- The power of a microscope can be described in terms of its **magnification** and **resolution**.
- Resolution can be increased by shortening wavelength, increasing the **numerical aperture** of the lens, or using stains that enhance contrast.

Short Answer

Exercise:

Problem:

Explain how a prism separates white light into different colors.

Critical Thinking

Exercise:

Problem: In [\[link\]](#), which of the following has the lowest energy?

- A. visible light
- B. X-rays
- C. ultraviolet rays
- D. infrared rays

Peering Into the Invisible World

LEARNING OBJECTIVES

- Describe historical developments and individual contributions that led to the invention and development of the microscope
- Compare and contrast the features of simple and compound microscopes
- Explain why microscopy is a major tool used by microbiologists

Some of the fundamental characteristics and functions of microscopes can be understood in the context of the history of their use. Italian scholar Girolamo Fracastoro is regarded as the first person to formally postulate that disease was spread by tiny invisible *seminaria*, or “seeds of the contagion.” In his book *De Contagione* (1546), he proposed that these seeds could attach themselves to certain objects (which he called *fomes* [cloth]) that supported their transfer from person to person. However, since the technology for seeing such tiny objects did not yet exist, the existence of the *seminaria* remained hypothetical for a little over a century—an invisible world waiting to be revealed.

Early Microscopes

Antonie van Leeuwenhoek, sometimes hailed as “the Father of Microbiology,” is typically credited as the first person to have created microscopes powerful enough to view microbes ([\[link\]](#)). Born in the city of Delft in the Dutch Republic, van Leeuwenhoek began his career selling fabrics. However, he later became interested in lens making (perhaps to

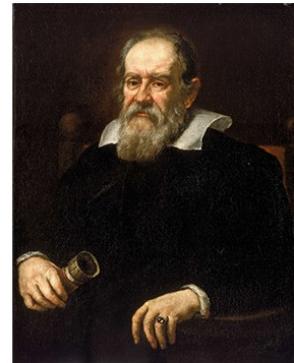
look at threads) and his innovative techniques produced microscopes that allowed him to observe microorganisms as no one had before. In 1674, he described his observations of single-celled organisms, whose existence was previously unknown, in a series of letters to the Royal Society of London. His report was initially met with skepticism, but his claims were soon verified and he became something of a celebrity in the scientific community.



(a)



(b)



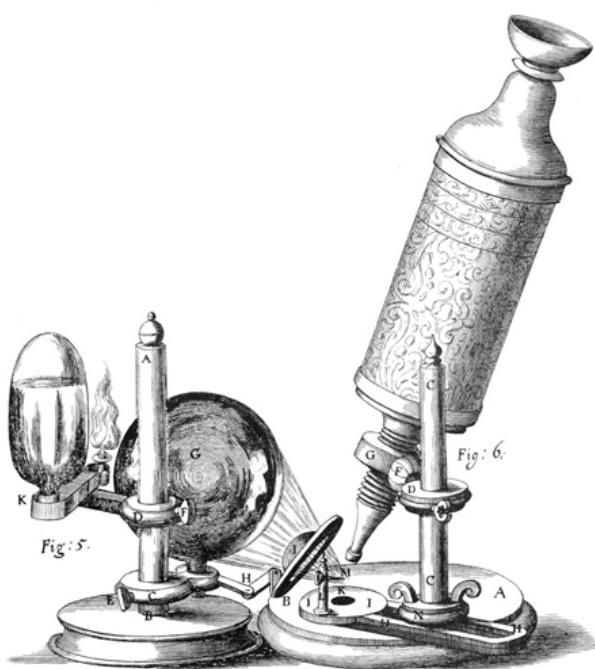
(c)

(a) Antonie van Leeuwenhoek (1632–1723) is credited as being the first person to observe microbes, including bacteria, which he called “animalcules” and “wee little beasties.” (b) Even though van Leeuwenhoek’s microscopes were simple microscopes (as seen in this replica), they were more powerful and provided better resolution than the compound microscopes of his day. (c) Though more famous for developing the telescope, Galileo Galilei (1564–1642) was also one of the pioneers of microscopy. (credit b: modification of work by “Wellcome Images”/Wikimedia Commons)

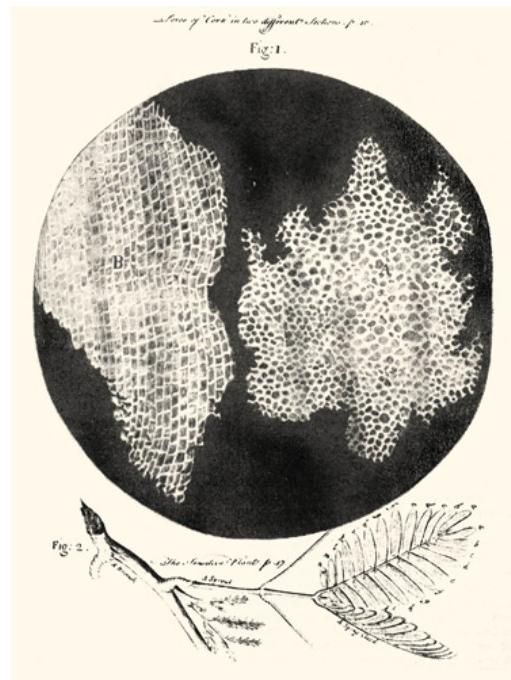
While van Leeuwenhoek is credited with the discovery of microorganisms, others before him had contributed to the development of the microscope. These included eyeglass makers in the Netherlands in the late 1500s, as well as the Italian astronomer Galileo Galilei, who used a **compound microscope** to examine insect parts ([\[link\]](#)). Whereas van Leeuwenhoek

used a **simple microscope**, in which light is passed through just one lens, Galileo's compound microscope was more sophisticated, passing light through two sets of lenses.

Van Leeuwenhoek's contemporary, the Englishman Robert Hooke (1635–1703), also made important contributions to microscopy, publishing in his book *Micrographia* (1665) many observations using compound microscopes. Viewing a thin sample of cork through his microscope, he was the first to observe the structures that we now know as cells ([\[link\]](#)). Hooke described these structures as resembling “Honey-comb,” and as “small Boxes or Bladders of Air,” noting that each “Cavern, Bubble, or Cell” is distinct from the others (in Latin, “cell” literally means “small room”). They likely appeared to Hooke to be filled with air because the cork cells were dead, with only the rigid cell walls providing the structure.



(a)



(b)

Robert Hooke used his (a) compound microscope to view (b) cork cells. Both of these engravings are from his seminal work *Micrographia*, published in 1665.

Note:

- Explain the difference between simple and compound microscopes.
- Compare and contrast the contributions of van Leeuwenhoek, Hooke, and Galileo to early microscopy.

Note:

Who Invented the Microscope?

While Antonie van Leeuwenhoek and Robert Hooke generally receive much of the credit for early advances in microscopy, neither can claim to be the inventor of the microscope. Some argue that this designation should belong to Hans and Zaccharias Janssen, Dutch spectacle-makers who may have invented the telescope, the simple microscope, and the compound microscope during the late 1500s or early 1600s ([\[link\]](#)). Unfortunately, little is known for sure about the Janssens, not even the exact dates of their births and deaths. The Janssens were secretive about their work and never published. It is also possible that the Janssens did not invent anything at all; their neighbor, Hans Lippershey, also developed microscopes and telescopes during the same time frame, and he is often credited with inventing the telescope. The historical records from the time are as fuzzy and imprecise as the images viewed through those early lenses, and any archived records have been lost over the centuries.

By contrast, van Leeuwenhoek and Hooke can thank ample documentation of their work for their respective legacies. Like Janssen, van Leeuwenhoek began his work in obscurity, leaving behind few records. However, his friend, the prominent physician Reinier de Graaf, wrote a letter to the editor of the *Philosophical Transactions of the Royal Society of London* calling attention to van Leeuwenhoek's powerful microscopes. From 1673 onward, van Leeuwenhoek began regularly submitting letters to the Royal Society detailing his observations. In 1674, his report describing single-

celled organisms produced controversy in the scientific community, but his observations were soon confirmed when the society sent a delegation to investigate his findings. He subsequently enjoyed considerable celebrity, at one point even entertaining a visit by the czar of Russia.

Similarly, Robert Hooke had his observations using microscopes published by the Royal Society in a book called *Micrographia* in 1665. The book became a bestseller and greatly increased interest in microscopy throughout much of Europe.



Zaccharias Janssen, along with his father Hans, may have invented the telescope, the simple microscope, and

the compound microscope during the late 1500s or early 1600s. The historical evidence is inconclusive.

Key Concepts and Summary

- **Antonie van Leeuwenhoek** is credited with the first observation of microbes, including protists and bacteria, with simple microscopes that he made.
- **Robert Hooke** was the first to describe what we now call cells.
- **Simple microscopes** have a single lens, while **compound microscopes** have multiple lenses.

Instruments of Microscopy

LEARNING OBJECTIVES

- Identify and describe the parts of a brightfield microscope
- Calculate total magnification for a compound microscope
- Describe the distinguishing features and typical uses for various types of light microscopes, electron microscopes, and scanning probe microscopes

The early pioneers of microscopy opened a window into the invisible world of microorganisms. But microscopy continued to advance in the centuries that followed. In 1830, Joseph Jackson Lister created an essentially modern light microscope. The 20th century saw the development of microscopes that leveraged nonvisible light, such as fluorescence microscopy, which uses an ultraviolet light source, and electron microscopy, which uses short-wavelength electron beams. These advances led to major improvements in magnification, resolution, and contrast. By comparison, the relatively rudimentary microscopes of van Leeuwenhoek and his contemporaries were far less powerful than even the most basic microscopes in use today. In this section, we will survey the broad range of modern microscopic technology and common applications for each type of microscope.

Light Microscopy

Many types of microscopes fall under the category of light microscopes, which use light to visualize images. Examples of light microscopes include brightfield microscopes, darkfield microscopes, phase-contrast microscopes, differential interference contrast microscopes, fluorescence

microscopes, confocal scanning laser microscopes, and two-photon microscopes. These various types of light microscopes can be used to complement each other in diagnostics and research.

Brightfield Microscopes

The **brightfield microscope**, perhaps the most commonly used type of microscope, is a compound microscope with two or more lenses that produce a dark image on a bright background. Some brightfield microscopes are **monocular** (having a single eyepiece), though most newer brightfield microscopes are **binocular** (having two eyepieces), like the one shown in [\[link\]](#); in either case, each eyepiece contains a lens called an **ocular lens**. The ocular lenses typically magnify images 10 times ($10\times$). At the other end of the body tube are a set of **objective lenses** on a rotating nosepiece. The magnification of these objective lenses typically ranges from $4\times$ to $100\times$, with the magnification for each lens designated on the metal casing of the lens. The ocular and objective lenses work together to create a magnified image. The **total magnification** is the product of the ocular magnification times the objective magnification:

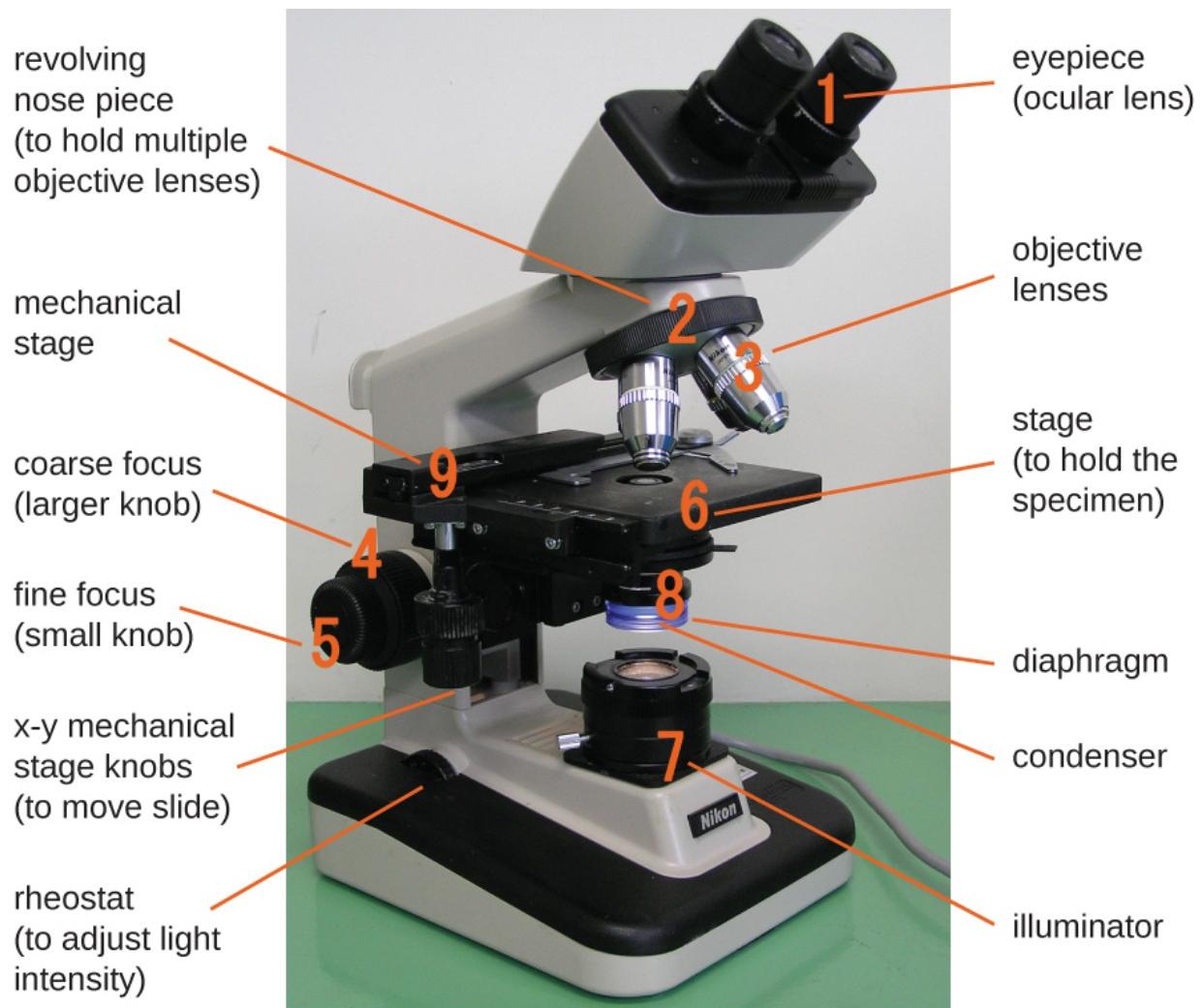
Equation:

$$\text{ocular magnification} \times \text{objective magnification}$$

For example, if a $40\times$ objective lens is selected and the ocular lens is $10\times$, the total magnification would be

Equation:

$$(40\times)(10\times) = 400\times$$



Components of a typical brightfield microscope.

The item being viewed is called a specimen. The specimen is placed on a glass slide, which is then clipped into place on the **stage** (a platform) of the microscope. Once the slide is secured, the specimen on the slide is positioned over the light using the **x-y mechanical stage knobs**. These knobs move the slide on the surface of the stage, but do not raise or lower the stage. Once the specimen is centered over the light, the stage position can be raised or lowered to focus the image. The **coarse focusing knob** is used for large-scale movements with $4\times$ and $10\times$ objective lenses; the **fine**

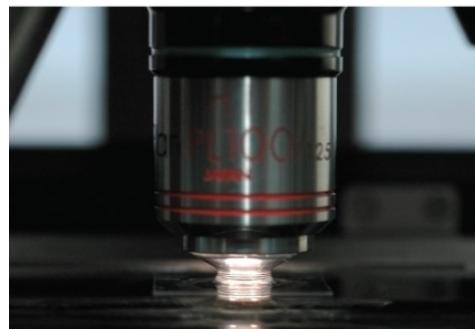
focusing knob is used for small-scale movements, especially with $40\times$ or $100\times$ objective lenses.

When images are magnified, they become dimmer because there is less light per unit area of image. Highly magnified images produced by microscopes, therefore, require intense lighting. In a brightfield microscope, this light is provided by an **illuminator**, which is typically a high-intensity bulb below the stage. Light from the illuminator passes up through **condenser lens** (located below the stage), which focuses all of the light rays on the specimen to maximize illumination. The position of the condenser can be optimized using the attached condenser focus knob; once the optimal distance is established, the condenser should not be moved to adjust the brightness. If less-than-maximal light levels are needed, the amount of light striking the specimen can be easily adjusted by opening or closing a **diaphragm** between the condenser and the specimen. In some cases, brightness can also be adjusted using the **rheostat**, a dimmer switch that controls the intensity of the illuminator.

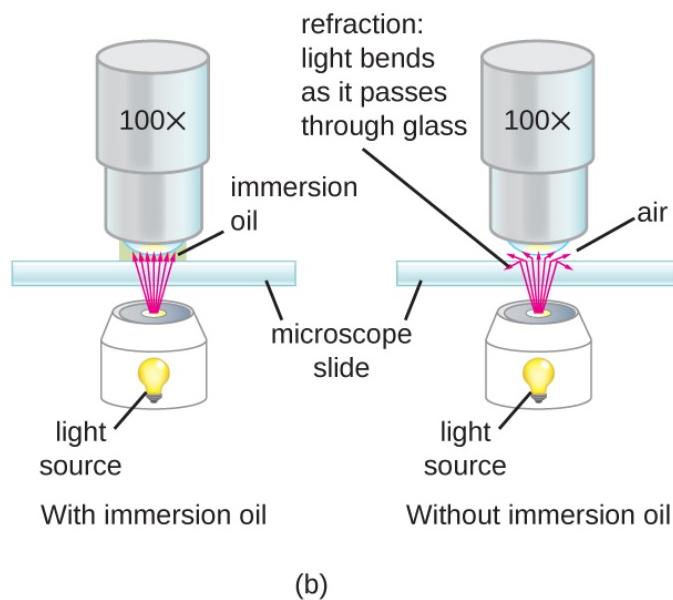
A brightfield microscope creates an image by directing light from the illuminator at the specimen; this light is differentially transmitted, absorbed, reflected, or refracted by different structures. Different colors can behave differently as they interact with **chromophores** (pigments that absorb and reflect particular wavelengths of light) in parts of the specimen. Often, chromophores are artificially added to the specimen using stains, which serve to increase contrast and resolution. In general, structures in the specimen will appear darker, to various extents, than the bright background, creating maximally sharp images at magnifications up to about $1000\times$. Further magnification would create a larger image, but without increased resolution. This allows us to see objects as small as bacteria, which are visible at about $400\times$ or so, but not smaller objects such as viruses.

At very high magnifications, resolution may be compromised when light passes through the small amount of air between the specimen and the lens. This is due to the large difference between the refractive indices of air and glass; the air scatters the light rays before they can be focused by the lens. To solve this problem, a drop of oil can be used to fill the space between the specimen and an **oil immersion lens**, a special lens designed to be used

with immersion oils. Since the oil has a refractive index very similar to that of glass, it increases the maximum angle at which light leaving the specimen can strike the lens. This increases the light collected and, thus, the resolution of the image ([\[link\]](#)). A variety of oils can be used for different types of light.



(a)



(b)

- (a) Oil immersion lenses like this one are used to improve resolution.
- (b) Because immersion oil and glass have very similar refractive indices, there is a minimal amount of refraction before the light reaches the lens. Without immersion oil, light scatters as it passes through the air above the slide, degrading the resolution of the image.

Note:

Microscope Maintenance: Best Practices

Even a very powerful microscope cannot deliver high-resolution images if it is not properly cleaned and maintained. Since lenses are carefully designed and manufactured to refract light with a high degree of precision, even a slightly dirty or scratched lens will refract light in unintended ways,

degrading the image of the specimen. In addition, microscopes are rather delicate instruments, and great care must be taken to avoid damaging parts and surfaces. Among other things, proper care of a microscope includes the following:

- cleaning the lenses with lens paper
- not allowing lenses to contact the slide (e.g., by rapidly changing the focus)
- protecting the bulb (if there is one) from breakage
- not pushing an objective into a slide
- not using the coarse focusing knob when using the $40\times$ or greater objective lenses
- only using immersion oil with a specialized oil objective, usually the $100\times$ objective
- cleaning oil from immersion lenses after using the microscope
- cleaning any oil accidentally transferred from other lenses
- covering the microscope or placing it in a cabinet when not in use

Note:



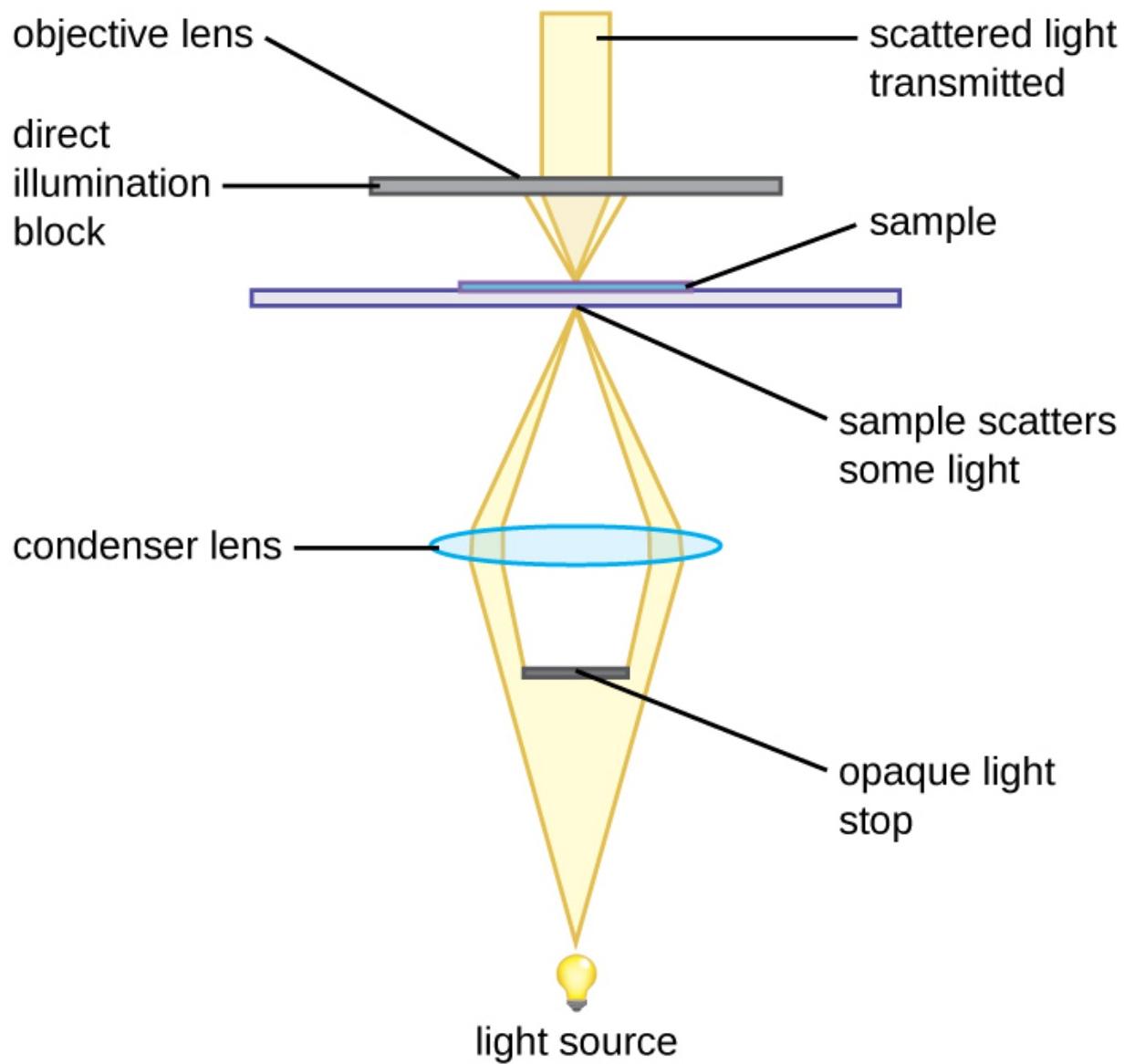
Visit the online resources linked below for simulations and demonstrations involving the use of microscopes. Keep in mind that execution of specific techniques and procedures can vary depending on the specific instrument you are using. Thus, it is important to learn and practice with an actual microscope in a laboratory setting under expert supervision.

- University of Delaware's [Virtual Microscope](#)

- St. John's University [Microscope Tutorials](#)

Darkfield Microscopy

A **darkfield microscope** is a brightfield microscope that has a small but significant modification to the condenser. A small, opaque disk (about 1 cm in diameter) is placed between the illuminator and the condenser lens. This opaque light stop, as the disk is called, blocks most of the light from the illuminator as it passes through the condenser on its way to the objective lens, producing a hollow cone of light that is focused on the specimen. The only light that reaches the objective is light that has been refracted or reflected by structures in the specimen. The resulting image typically shows bright objects on a dark background ([\[link\]](#)).



An opaque light stop inserted into a brightfield microscope is used to produce a darkfield image. The light stop blocks light traveling directly from the illuminator to the objective lens, allowing only light reflected or refracted off the specimen to reach the eye.

Darkfield microscopy can often create high-contrast, high-resolution images of specimens without the use of stains, which is particularly useful for viewing live specimens that might be killed or otherwise compromised by the stains. For example, thin spirochetes like *Treponema pallidum*, the

causative agent of syphilis, can be best viewed using a darkfield microscope ([\[link\]](#)).



Use of a darkfield microscope allows us to view living, unstained samples of the spirochete *Treponema pallidum*.

Similar to a photographic negative, the spirochetes appear bright against a dark background. (credit: Centers for Disease Control and Prevention)

Note:

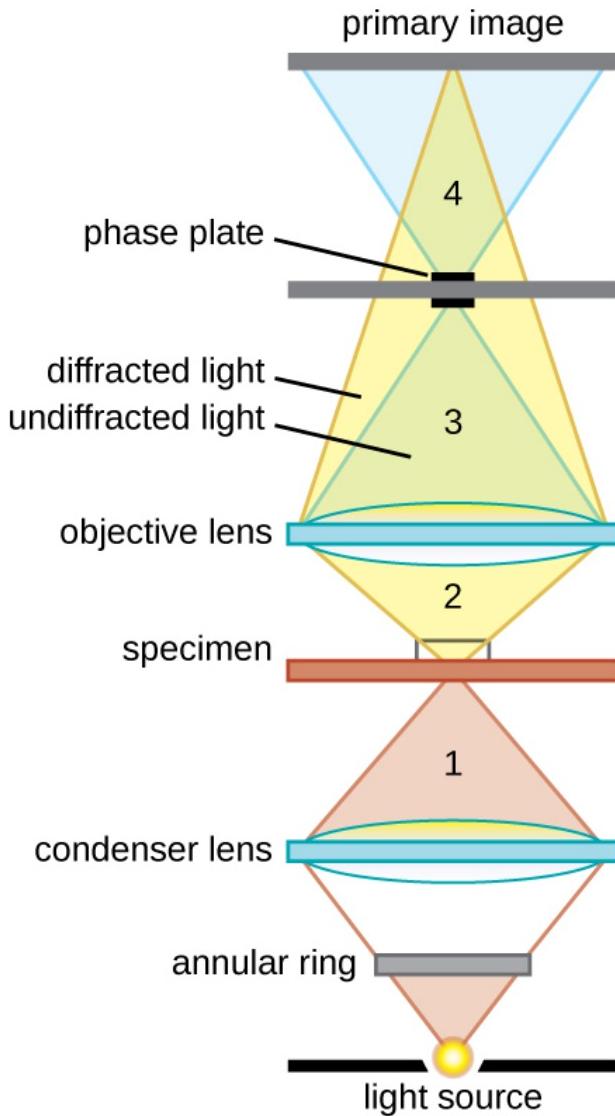
- Identify the key differences between brightfield and darkfield microscopy.

Phase-Contrast Microscopes

Phase-contrast microscopes use refraction and interference caused by structures in a specimen to create high-contrast, high-resolution images without staining. It is the oldest and simplest type of microscope that creates an image by altering the wavelengths of light rays passing through the specimen. To create altered wavelength paths, an annular stop is used in the condenser. The annular stop produces a hollow cone of light that is focused on the specimen before reaching the objective lens. The objective contains a phase plate containing a phase ring. As a result, light traveling directly from the illuminator passes through the phase ring while light refracted or reflected by the specimen passes through the plate. This causes waves traveling through the ring to be about one-half of a wavelength out of phase with those passing through the plate. Because waves have peaks and troughs, they can add together (if in phase together) or cancel each other out (if out of phase). When the wavelengths are out of phase, wave troughs will cancel out wave peaks, which is called destructive interference. Structures that refract light then appear dark against a bright background of only unrefracted light. More generally, structures that differ in features such as refractive index will differ in levels of darkness ([\[link\]](#)).

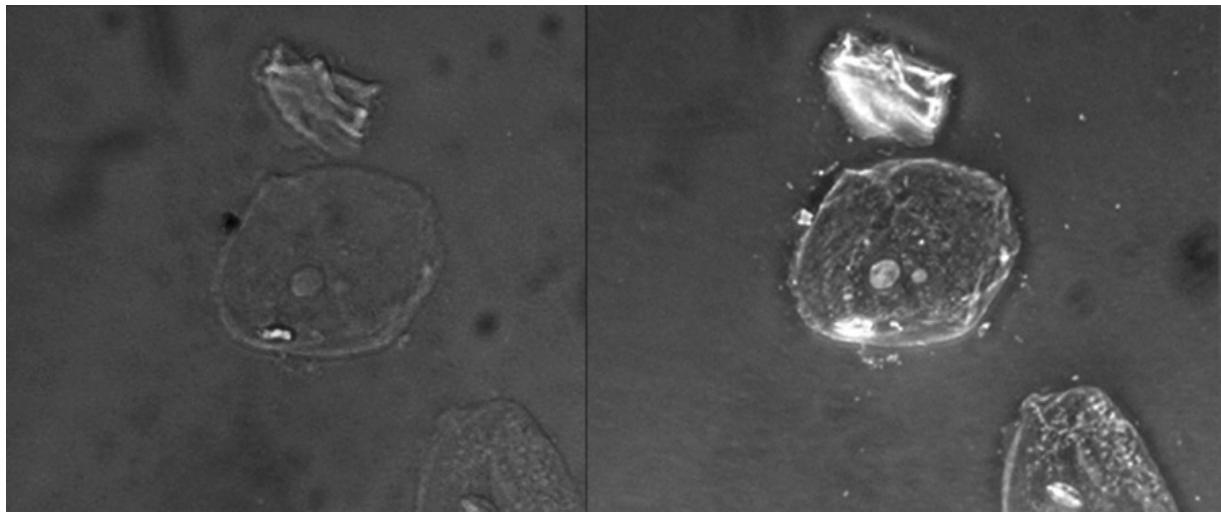
- 4 Wavelengths in phase or out of phase either add together or cancel out each other.
- 3 Light traveling directly from the condenser lens and light traveling through the specimen are out of phase when they pass through the objective and phase plates.
- 2 Object or specimen refracts or reflects light.
- 1 Annular stop in the condenser produces a cone of light focused on the specimen.

- Illuminating light
- Diffracted light
- Undiffracted light
- Combined diffracted and undiffracted light



This diagram of a phase-contrast microscope illustrates phase differences between light passing through the object and background. These differences are produced by passing the rays through different parts of a phase plate. The light rays are superimposed in the image plane, producing contrast due to their interference.

Because it increases contrast without requiring stains, phase-contrast microscopy is often used to observe live specimens. Certain structures, such as organelles in eukaryotic cells and endospores in prokaryotic cells, are especially well visualized with phase-contrast microscopy ([\[link\]](#)).

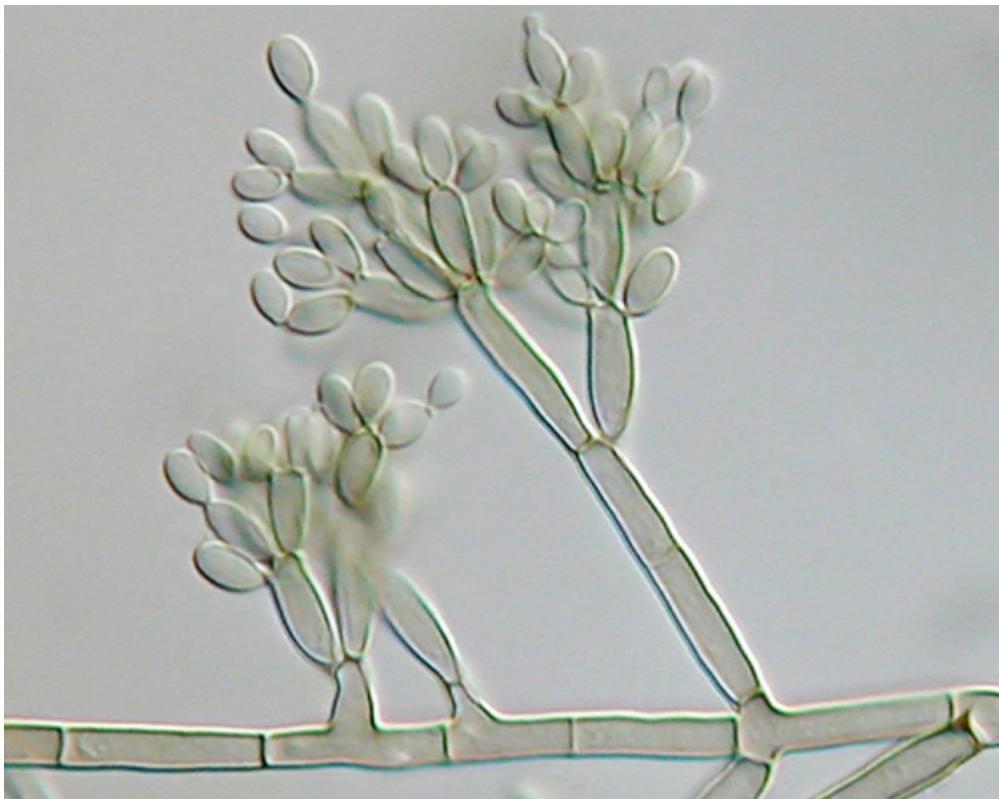


This figure compares a brightfield image (left) with a phase-contrast image (right) of the same unstained simple squamous epithelial cells.

The cells are in the center and bottom right of each photograph (the irregular item above the cells is acellular debris). Notice that the unstained cells in the brightfield image are almost invisible against the background, whereas the cells in the phase-contrast image appear to glow against the background, revealing far more detail.

Differential Interference Contrast Microscopes

Differential interference contrast (DIC) microscopes (also known as Nomarski optics) are similar to phase-contrast microscopes in that they use interference patterns to enhance contrast between different features of a specimen. In a DIC microscope, two beams of light are created in which the direction of wave movement (polarization) differs. Once the beams pass through either the specimen or specimen-free space, they are recombined and effects of the specimens cause differences in the interference patterns generated by the combining of the beams. This results in high-contrast images of living organisms with a three-dimensional appearance. These microscopes are especially useful in distinguishing structures within live, unstained specimens. ([\[link\]](#))



A DIC image of *Fonsecaea pedrosoi* grown on modified Leonian's agar. This fungus causes chromoblastomycosis, a chronic skin infection common in tropical and subtropical climates.

Note:

- What are some advantages of phase-contrast and DIC microscopy?

Fluorescence Microscopes

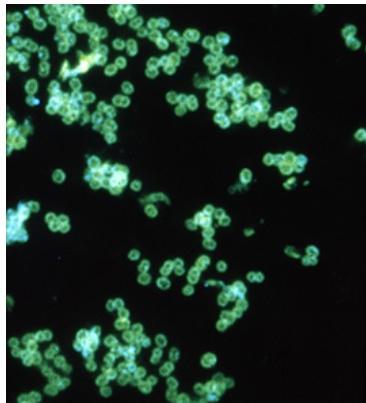
A **fluorescence microscope** uses fluorescent chromophores called **fluorochromes**, which are capable of absorbing energy from a light source and then emitting this energy as visible light. Fluorochromes include naturally fluorescent substances (such as chlorophylls) as well as fluorescent stains that are added to the specimen to create contrast. Dyes such as Texas red and FITC are examples of fluorochromes. Other examples include the nucleic acid dyes 4',6'-diamidino-2-phenylindole (DAPI) and acridine orange.

The microscope transmits an excitation light, generally a form of EMR with a short wavelength, such as ultraviolet or blue light, toward the specimen; the chromophores absorb the excitation light and emit visible light with longer wavelengths. The excitation light is then filtered out (in part because ultraviolet light is harmful to the eyes) so that only visible light passes through the ocular lens. This produces an image of the specimen in bright colors against a dark background.

Fluorescence microscopes are especially useful in clinical microbiology. They can be used to identify pathogens, to find particular species within an environment, or to find the locations of particular molecules and structures within a cell. Approaches have also been developed to distinguish living from dead cells using fluorescence microscopy based upon whether they take up particular fluorochromes. Sometimes, multiple fluorochromes are used on the same specimen to show different structures or features.

One of the most important applications of fluorescence microscopy is a technique called **immunofluorescence**, which is used to identify certain disease-causing microbes by observing whether antibodies bind to them. (Antibodies are protein molecules produced by the immune system that attach to specific pathogens to kill or inhibit them.) There are two approaches to this technique: direct immunofluorescence assay (DFA) and indirect immunofluorescence assay (IFA). In DFA, specific antibodies (e.g., those that target the rabies virus) are stained with a fluorochrome. If the specimen contains the targeted pathogen, one can observe the antibodies binding to the pathogen under the fluorescent microscope. This is called a primary antibody stain because the stained antibodies attach directly to the pathogen.

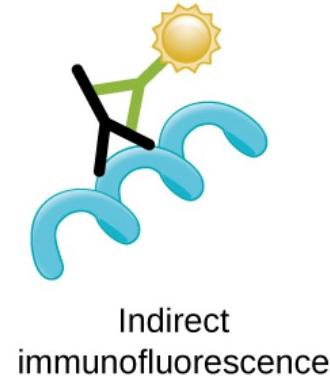
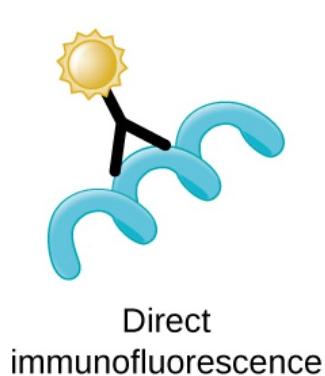
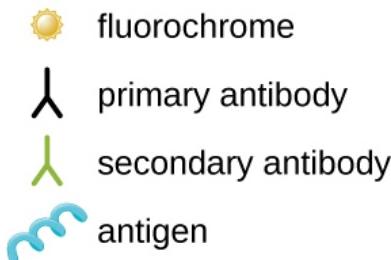
In IFA, secondary antibodies are stained with a fluorochrome rather than primary antibodies. Secondary antibodies do not attach directly to the pathogen, but they do bind to primary antibodies. When the unstained primary antibodies bind to the pathogen, the fluorescent secondary antibodies can be observed binding to the primary antibodies. Thus, the secondary antibodies are attached indirectly to the pathogen. Since multiple secondary antibodies can often attach to a primary antibody, IFA increases the number of fluorescent antibodies attached to the specimen, making it easier to visualize features in the specimen ([\[link\]](#)).



(a)



(b)



(c)

(a) A direct immunofluorescent stain is used to visualize *Neisseria gonorrhoeae*, the bacterium that causes gonorrhea. (b) An indirect immunofluorescent stain is used to visualize larvae of *Schistosoma*

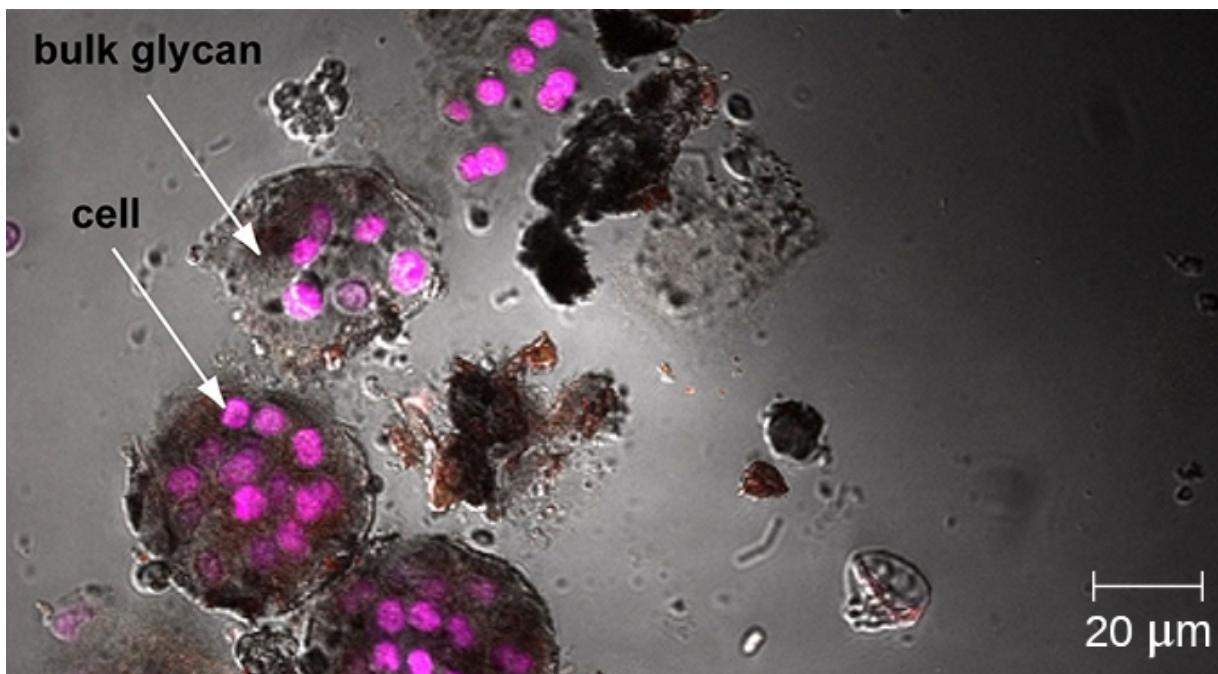
mansoni, a parasitic worm that causes schistosomiasis, an intestinal disease common in the tropics. (c) In direct immunofluorescence, the stain is absorbed by a primary antibody, which binds to the antigen. In indirect immunofluorescence, the stain is absorbed by a secondary antibody, which binds to a primary antibody, which, in turn, binds to the antigen. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by Centers for Disease Control and Prevention)

Note:

- Why must fluorochromes be used to examine a specimen under a fluorescence microscope?

Confocal Microscopes

Whereas other forms of light microscopy create an image that is maximally focused at a single distance from the observer (the depth, or z-plane), a **confocal microscope** uses a laser to scan multiple z-planes successively. This produces numerous two-dimensional, high-resolution images at various depths, which can be constructed into a three-dimensional image by a computer. As with fluorescence microscopes, fluorescent stains are generally used to increase contrast and resolution. Image clarity is further enhanced by a narrow aperture that eliminates any light that is not from the z-plane. Confocal microscopes are thus very useful for examining thick specimens such as biofilms, which can be examined alive and unfixed ([\[link\]](#)).



Confocal microscopy can be used to visualize structures such as this roof-dwelling cyanobacterium biofilm. (credit: modification of work by American Society for Microbiology)

Note:



Explore a rotating three-dimensional [view](#) of a biofilm as observed under a confocal microscope. After navigating to the webpage, click the “play” button to launch the video.

Two-Photon Microscopes

While the original fluorescent and confocal microscopes allowed better visualization of unique features in specimens, there were still problems that prevented optimum visualization. The effective sensitivity of fluorescence microscopy when viewing thick specimens was generally limited by out-of-focus flare, which resulted in poor resolution. This limitation was greatly reduced in the confocal microscope through the use of a confocal pinhole to reject out-of-focus background fluorescence with thin ($<1\text{ }\mu\text{m}$), unblurred optical sections. However, even the confocal microscopes lacked the resolution needed for viewing thick tissue samples. These problems were resolved with the development of the **two-photon microscope**, which uses a scanning technique, fluorochromes, and long-wavelength light (such as infrared) to visualize specimens. The low energy associated with the long-wavelength light means that two photons must strike a location at the same time to excite the fluorochrome. The low energy of the excitation light is less damaging to cells, and the long wavelength of the excitation light more easily penetrates deep into thick specimens. This makes the two-photon microscope useful for examining living cells within intact tissues—brain slices, embryos, whole organs, and even entire animals.

Currently, use of two-photon microscopes is limited to advanced clinical and research laboratories because of the high costs of the instruments. A single two-photon microscope typically costs between \$300,000 and \$500,000, and the lasers used to excite the dyes used on specimens are also very expensive. However, as technology improves, two-photon microscopes may become more readily available in clinical settings.

Note:

- What types of specimens are best examined using confocal or two-photon microscopy?

Electron Microscopy

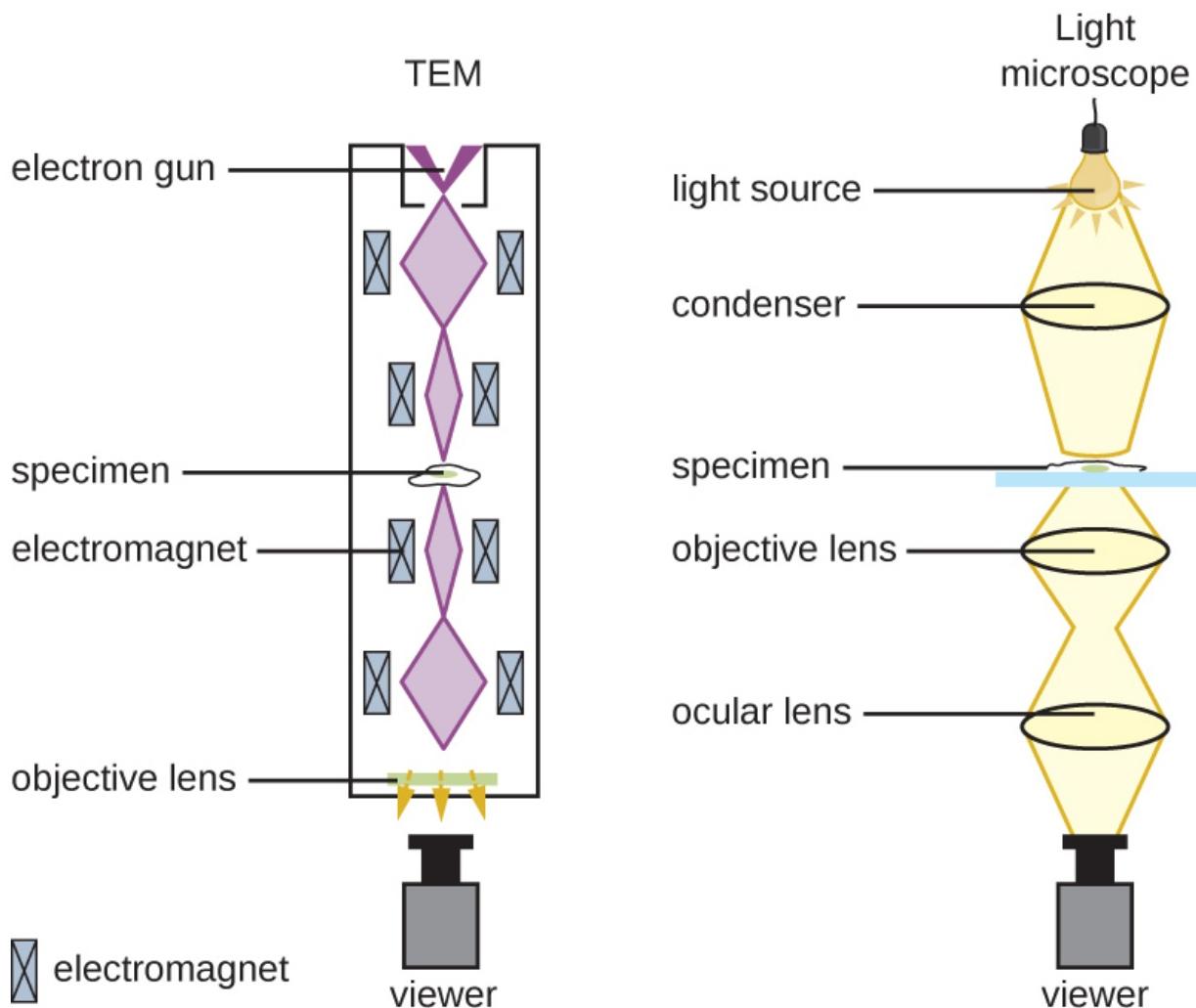
The maximum theoretical resolution of images created by light microscopes is ultimately limited by the wavelengths of visible light. Most light microscopes can only magnify $1000\times$, and a few can magnify up to $1500\times$, but this does not begin to approach the magnifying power of an **electron microscope (EM)**, which uses short-wavelength electron beams rather than light to increase magnification and resolution.

Electrons, like electromagnetic radiation, can behave as waves, but with wavelengths of 0.005 nm, they can produce much better resolution than visible light. An EM can produce a sharp image that is magnified up to $100,000\times$. Thus, EMs can resolve subcellular structures as well as some molecular structures (e.g., single strands of DNA); however, electron microscopy cannot be used on living material because of the methods needed to prepare the specimens.

There are two basic types of EM: the **transmission electron microscope (TEM)** and the **scanning electron microscope (SEM)** ([\[link\]](#)). The TEM is somewhat analogous to the brightfield light microscope in terms of the way it functions. However, it uses an electron beam from above the specimen that is focused using a magnetic lens (rather than a glass lens) and projected through the specimen onto a detector. Electrons pass through the specimen, and then the detector captures the image ([\[link\]](#)).



A transmission electron microscope (TEM).

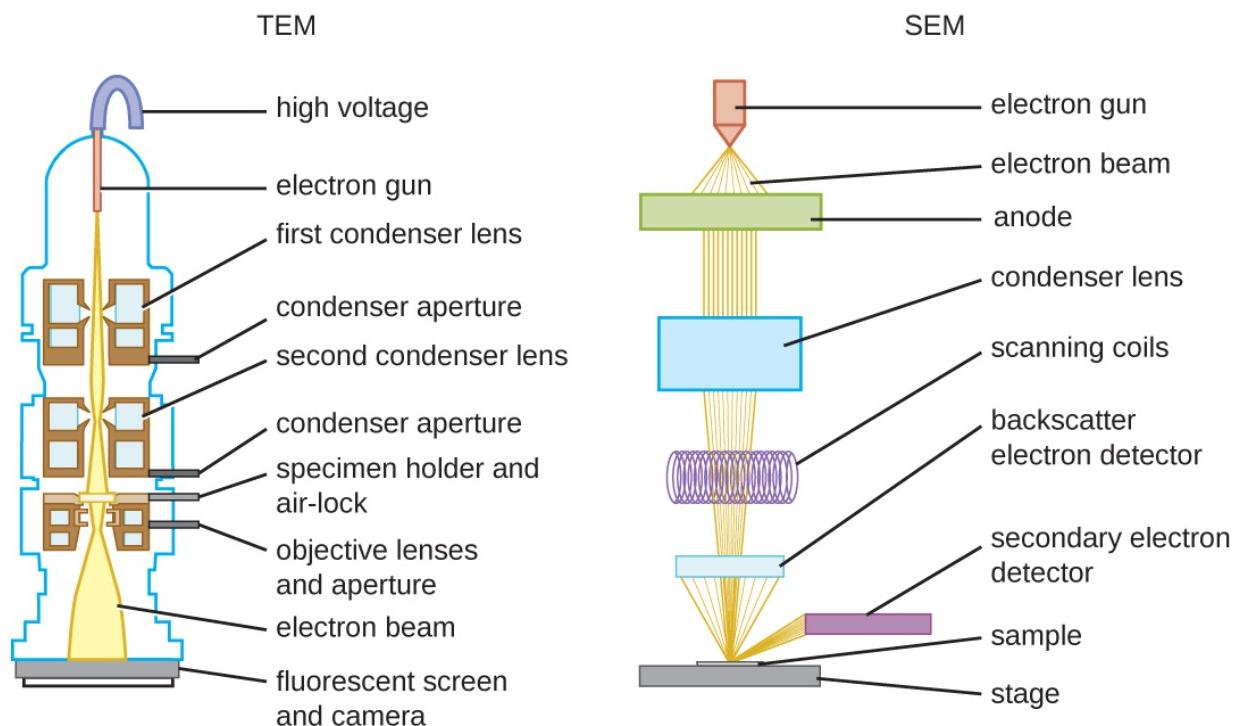


Electron microscopes use magnets to focus electron beams similarly to the way that light microscopes use lenses to focus light.

For electrons to pass through the specimen in a TEM, the specimen must be extremely thin (20–100 nm thick). The image is produced because of varying opacity in various parts of the specimen. This opacity can be enhanced by staining the specimen with materials such as heavy metals, which are electron dense. TEM requires that the beam and specimen be in a vacuum and that the specimen be very thin and dehydrated. The specific steps needed to prepare a specimen for observation under an EM are discussed in detail in the next section.

SEMs form images of surfaces of specimens, usually from electrons that are knocked off of specimens by a beam of electrons. This can create highly detailed images with a three-dimensional appearance that are displayed on a monitor ([\[link\]](#)). Typically, specimens are dried and prepared with fixatives that reduce artifacts, such as shriveling, that can be produced by drying, before being sputter-coated with a thin layer of metal such as gold. Whereas transmission electron microscopy requires very thin sections and allows one to see internal structures such as organelles and the interior of membranes, scanning electron microscopy can be used to view the surfaces of larger objects (such as a pollen grain) as well as the surfaces of very small samples ([\[link\]](#)). Some EMs can magnify an image up to 2,000,000 \times . [\[footnote\]](#)

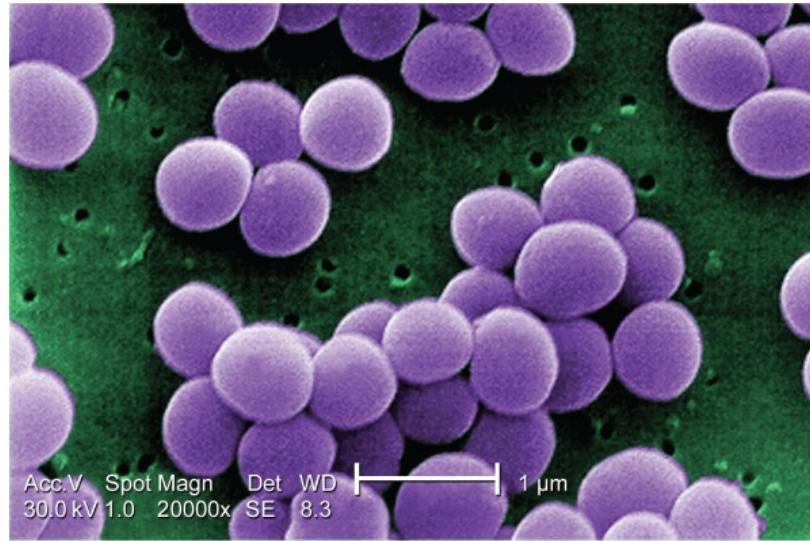
“JEM-ARM200F Transmission Electron Microscope,” *JEOL USA Inc*, <http://www.jeolusa.com/PRODUCTS/TransmissionElectronMicroscopes%20TEM%29/200kV/JEM-ARM200F/tabid/663/Default.aspx#195028>- specifications. Accessed 8/28/2015.



These schematic illustrations compare the components of transmission electron microscopes and scanning electron microscopes.



(a)



(b)

(a) This TEM image of cells in a biofilm shows well-defined internal structures of the cells because of varying levels of opacity in the specimen. (b) This color-enhanced SEM image of the bacterium *Staphylococcus aureus* illustrates the ability of scanning electron microscopy to render three-dimensional images of the surface structure of cells. (credit a: modification of work by American Society for Microbiology; credit b: modification of work by Centers for Disease Control and Prevention)

Note:

- What are some advantages and disadvantages of electron microscopy, as opposed to light microscopy, for examining microbiological specimens?

- What kinds of specimens are best examined using TEM? SEM?

Note:

Using Microscopy to Study Biofilms

A biofilm is a complex community of one or more microorganism species, typically forming as a slimy coating attached to a surface because of the production of an extrapolymeric substance (EPS) that attaches to a surface or at the interface between surfaces (e.g., between air and water). In nature, biofilms are abundant and frequently occupy complex niches within ecosystems ([\[link\]](#)). In medicine, biofilms can coat medical devices and exist within the body. Because they possess unique characteristics, such as increased resistance against the immune system and to antimicrobial drugs, biofilms are of particular interest to microbiologists and clinicians alike. Because biofilms are thick, they cannot be observed very well using light microscopy; slicing a biofilm to create a thinner specimen might kill or disturb the microbial community. Confocal microscopy provides clearer images of biofilms because it can focus on one z-plane at a time and produce a three-dimensional image of a thick specimen. Fluorescent dyes can be helpful in identifying cells within the matrix. Additionally, techniques such as immunofluorescence and fluorescence in situ hybridization (FISH), in which fluorescent probes are used to bind to DNA, can be used.

Electron microscopy can be used to observe biofilms, but only after dehydrating the specimen, which produces undesirable artifacts and distorts the specimen. In addition to these approaches, it is possible to follow water currents through the shapes (such as cones and mushrooms) of biofilms, using video of the movement of fluorescently coated beads ([\[link\]](#)).

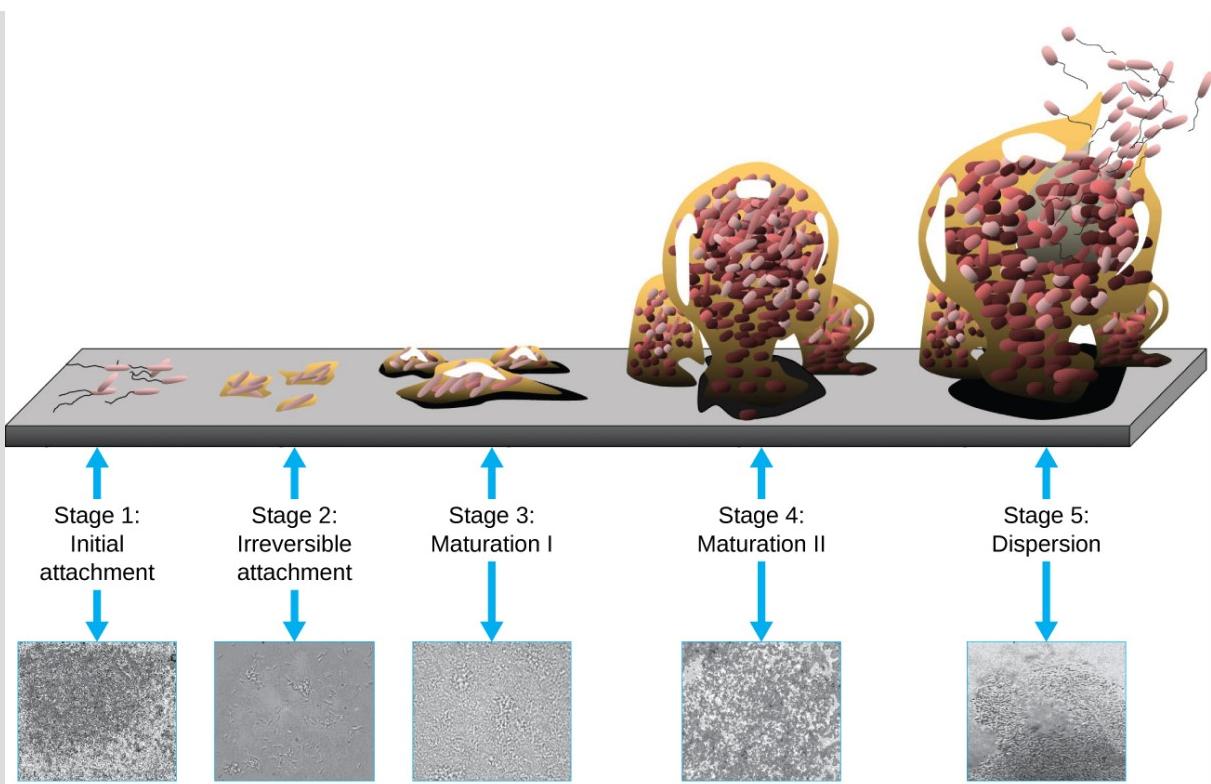
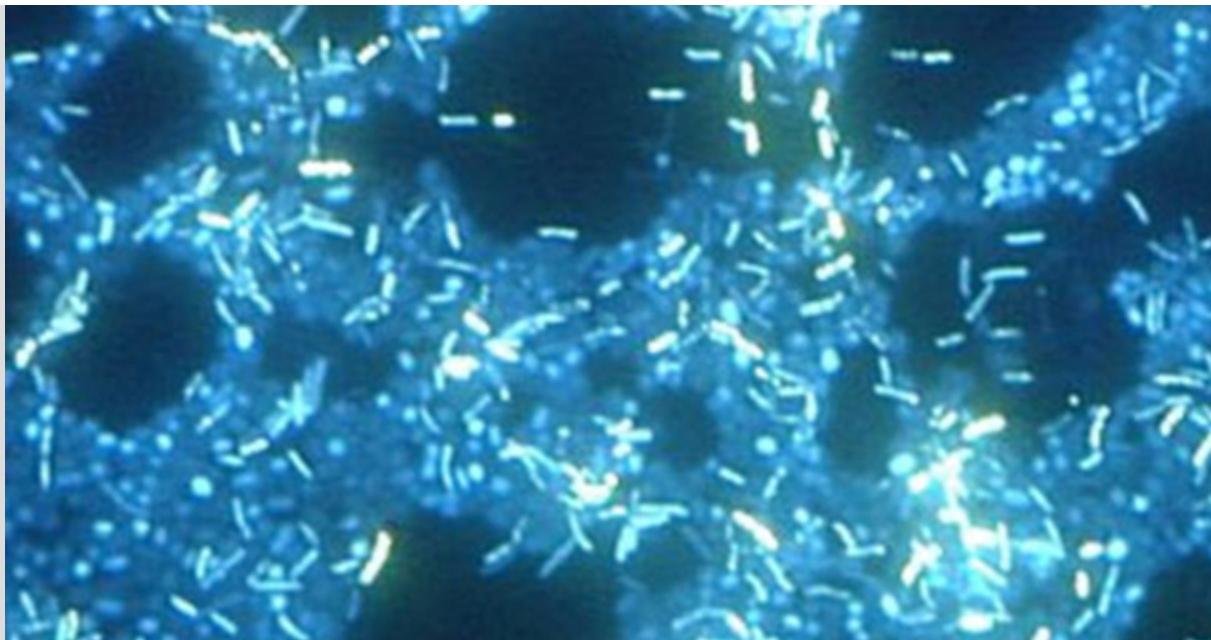


Diagram showing five stages of biofilm development of *Pseudomonas aeruginosa*. All photomicrographs are shown to same scale.

A biofilm forms when planktonic (free-floating) bacteria of one or more species adhere to a surface, produce slime, and form a colony.

(credit: Public Library of Science)



In this image, multiple species of bacteria grow in a biofilm on stainless steel (stained with DAPI for epifluorescence microscopy).

(credit: Ricardo Murga, Rodney Donlan)

Scanning Probe Microscopy

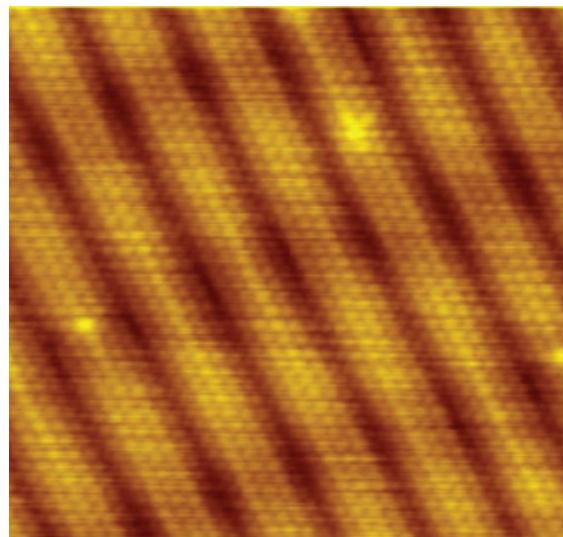
A **scanning probe microscope** does not use light or electrons, but rather very sharp probes that are passed over the surface of the specimen and interact with it directly. This produces information that can be assembled into images with magnifications up to $100,000,000\times$. Such large magnifications can be used to observe individual atoms on surfaces. To date, these techniques have been used primarily for research rather than for diagnostics.

There are two types of scanning probe microscope: the **scanning tunneling microscope (STM)** and the **atomic force microscope (AFM)**. An STM uses a probe that is passed just above the specimen as a constant voltage bias creates the potential for an electric current between the probe and the

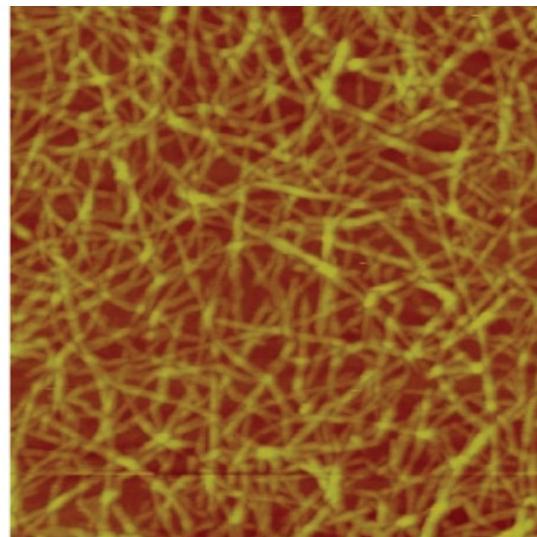
specimen. This current occurs via quantum tunneling of electrons between the probe and the specimen, and the intensity of the current is dependent upon the distance between the probe and the specimen. The probe is moved horizontally above the surface and the intensity of the current is measured. Scanning tunneling microscopy can effectively map the structure of surfaces at a resolution at which individual atoms can be detected.

Similar to an STM, AFMs have a thin probe that is passed just above the specimen. However, rather than measuring variations in the current at a constant height above the specimen, an AFM establishes a constant current and measures variations in the height of the probe tip as it passes over the specimen. As the probe tip is passed over the specimen, forces between the atoms (van der Waals forces, capillary forces, chemical bonding, electrostatic forces, and others) cause it to move up and down. Deflection of the probe tip is determined and measured using Hooke's law of elasticity, and this information is used to construct images of the surface of the specimen with resolution at the atomic level ([\[link\]](#)).

[\[link\]](#), [\[link\]](#), and [\[link\]](#) summarize the microscopy techniques for light microscopes, electron microscopes, and scanning probe microscopes, respectively.



(a)



(b)

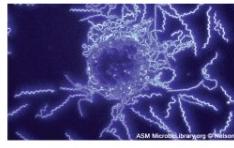
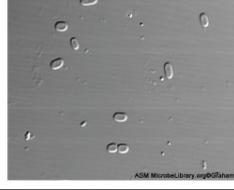
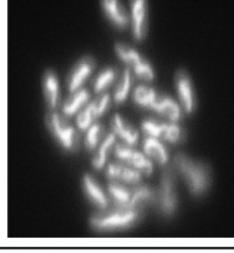
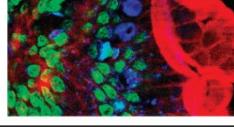
STMs and AFMs allow us to view images at the atomic level. (a) This STM image of a pure gold surface shows individual atoms of gold arranged in columns. (b) This AFM image shows long, strand-like molecules of nanocellulose, a laboratory-created substance derived from plant fibers. (credit a: modification of work by “Erwinrossen”/Wikimedia Commons)

Note:

- Which has higher magnification, a light microscope or a scanning probe microscope?
- Name one advantage and one limitation of scanning probe microscopy.

LIGHT MICROSCOPES Magnification: up to about 1000×

Use visible or ultraviolet light to produce an image.

Microscope Type	Key Uses	Sample Images
Brightfield	Commonly used in a wide variety of laboratory applications as the standard microscope; produces an image on a bright background. Example: <i>Bacillus</i> sp. showing endospores.	 ASM MicrobeLibrary.org©Smith
Darkfield	Increases contrast without staining by producing a bright image on a darker background; especially useful for viewing live specimens. Example: <i>Borrelia burgdorferi</i>	 ASM MicrobeLibrary.org©Nelson
Phase contrast	Uses refraction and interference caused by structures in the specimen to create high-contrast, high-resolution images without staining, making it useful for viewing live specimens, and structures such as endospores and organelles. Example: <i>Pseudomonas</i> sp.	 ASM MicrobeLibrary.org©Smith
Differential interference contrast (DIC)	Uses interference patterns to enhance contrast between different features of a specimen to produce high-contrast images of living organisms with a three-dimensional appearance, making it especially useful in distinguishing structures within live, unstained specimens; images viewed reveal detailed structures within cells. Example: <i>Escherichia coli</i> O157:H7	 ASM MicrobeLibrary.org©Smith
Fluorescence	Uses fluorescent stains to produce an image; can be used to identify pathogens, to find particular species, to distinguish living from dead cells, or to find locations of particular molecules within a cell; also used for immunofluorescence. Example: <i>P. putida</i> stained with fluorescent dyes to visualize the capsule.	 ASM MicrobeLibrary.org©Ghiorse
Confocal	Uses a laser to scan multiple z-planes successively, producing numerous two-dimensional, high-resolution images at various depths that can be constructed into a three-dimensional image by a computer, making this useful for examining thick specimens such as biofilms. Example: <i>Escherichia coli</i> stained with acridine orange dye to show the nucleoid regions of the cells.	 ASM MicrobeLibrary.org©Smith
Two-photon	Uses a scanning technique, fluorochromes, and long-wavelength light (such as infrared) to penetrate deep into thick specimens such as biofilms. Example: Mouse intestine cells stained with fluorescent dye.	 ASM MicrobeLibrary.org©Smith

(credit “Brightfield”: modification of work by American Society for Microbiology; credit “Darkfield”: modification of work by American Society for Microbiology; credit “Phase contrast”: modification of work by American Society for Microbiology; credit “DIC”:

modification of work by American Society for Microbiology; credit “Fluorescence”: modification of work by American Society for Microbiology; credit “Confocal”: modification of work by American Society for Microbiology; credit “Two-photon”: modification of work by Alberto Diaspro, Paolo Bianchini, Giuseppe Vicidomini, Mario Faretta, Paola Ramoino, Cesare Usai)

ELECTRON MICROSCOPES Magnification: 20–100,000\times or more Use electron beams focused with magnets to produce an image.		
Microscope Type	Key Uses	Sample Images
Transmission (TEM)	Uses electron beams that pass through a specimen to visualize small images; useful to observe small, thin specimens such as tissue sections and subcellular structures. Example: <i>Ebola virus</i>	
Scanning (SEM)	Uses electron beams to visualize surfaces; useful to observe the three-dimensional surface details of specimens. Example: <i>Campylobacter jejuni</i>	 2 μ m ASM MicrobeLibrary.org © Luton, Pfeil and Threadgill

(credit “TEM”: modification of work by American Society for Microbiology; credit “SEM”: modification of work by American Society for Microbiology)

SCANNING PROBE MICROSCOPES		Magnification: 100–100,000,000× or more
Use very short probes that are passed over the surface of the specimen and interact with it directly.		
Microscope Type	Key Uses	Sample Images
Scanning tunneling (STM)	<p>Uses a probe passed horizontally at a constant distance just above the specimen while the intensity of the current is measured; can map the structure of surfaces at the atomic level; works best on conducting materials but can also be used to examine organic materials such as DNA, if fixed on a surface.</p> <p>Example: Image of surface reconstruction on a clean gold [Au(100)] surface, as visualized using scanning tunneling microscopy.</p>	
Atomic force (AFM)	<p>Can be used in several ways, including using a laser focused on a cantilever to measure the bending of the tip or a probe passed above the specimen while the height needed to maintain a constant current is measured; useful to observe specimens at the atomic level and can be more easily used with nonconducting samples.</p> <p>Example: AFM height image of carboxymethylated nanocellulose adsorbed on a silica surface.</p>	

Key Concepts and Summary

- Numerous types of microscopes use various technologies to generate micrographs. Most are useful for a particular type of specimen or application.
- **Light microscopy** uses lenses to focus light on a specimen to produce an image. Commonly used light microscopes include **brightfield**, **darkfield**, **phase-contrast**, **differential interference contrast**, **fluorescence**, **confocal**, and **two-photon** microscopes.
- **Electron microscopy** focuses electrons on the specimen using magnets, producing much greater magnification than light microscopy. The **transmission electron microscope (TEM)** and **scanning electron microscope (SEM)** are two common forms.
- **Scanning probe microscopy** produces images of even greater magnification by measuring feedback from sharp probes that interact with the specimen. Probe microscopes include the **scanning tunneling microscope (STM)** and the **atomic force microscope (AFM)**.

Critical Thinking

Exercise:

Problem:

When focusing a light microscope, why is it best to adjust the focus using the coarse focusing knob before using the fine focusing knob?

Exercise:

Problem:

You need to identify structures within a cell using a microscope. However, the image appears very blurry even though you have a high magnification. What are some things that you could try to improve the resolution of the image? Describe the most basic factors that affect resolution when you first put the slide onto the stage; then consider more specific factors that could affect resolution for $40\times$ and $100\times$ lenses.

Staining Microscopic Specimens

LEARNING OBJECTIVES

- Differentiate between simple and differential stains
- Describe the unique features of commonly used stains
- Explain the procedures and name clinical applications for Gram, endospore, acid-fast, negative capsule, and flagella staining

In their natural state, most of the cells and microorganisms that we observe under the microscope lack color and contrast. This makes it difficult, if not impossible, to detect important cellular structures and their distinguishing characteristics without artificially treating specimens. We have already alluded to certain techniques involving stains and fluorescent dyes, and in this section we will discuss specific techniques for sample preparation in greater detail. Indeed, numerous methods have been developed to identify specific microbes, cellular structures, DNA sequences, or indicators of infection in tissue samples, under the microscope. Here, we will focus on the most clinically relevant techniques.

Preparing Specimens for Light Microscopy

In clinical settings, light microscopes are the most commonly used microscopes. There are two basic types of preparation used to view specimens with a light microscope: wet mounts and fixed specimens.

The simplest type of preparation is the **wet mount**, in which the specimen is placed on the slide in a drop of liquid. Some specimens, such as a drop of

urine, are already in a liquid form and can be deposited on the slide using a dropper. Solid specimens, such as a skin scraping, can be placed on the slide before adding a drop of liquid to prepare the wet mount. Sometimes the liquid used is simply water, but often stains are added to enhance contrast. Once the liquid has been added to the slide, a coverslip is placed on top and the specimen is ready for examination under the microscope.

The second method of preparing specimens for light microscopy is **fixation**. The “fixing” of a sample refers to the process of attaching cells to a slide. Fixation is often achieved either by heating (heat fixing) or chemically treating the specimen. In addition to attaching the specimen to the slide, fixation also kills microorganisms in the specimen, stopping their movement and metabolism while preserving the integrity of their cellular components for observation.

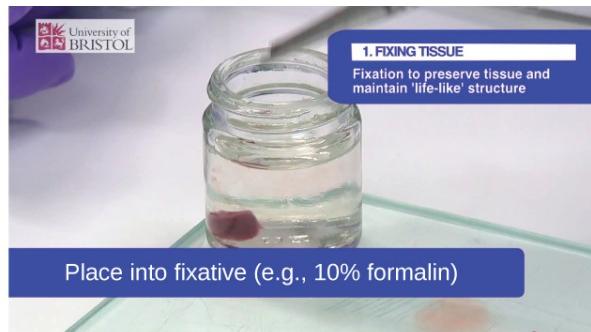
To heat-fix a sample, a thin layer of the specimen is spread on the slide (called a **smear**), and the slide is then briefly heated over a heat source ([\[link\]](#)). Chemical fixatives are often preferable to heat for tissue specimens. Chemical agents such as acetic acid, ethanol, methanol, formaldehyde (formalin), and glutaraldehyde can denature proteins, stop biochemical reactions, and stabilize cell structures in tissue samples ([\[link\]](#)).



(a)



(b)



(c)

- (a) A specimen can be heat-fixed by using a slide warmer like this one.
- (b) Another method for heat-fixing a specimen is to hold a slide with a smear over a microincinerator.
- (c) This tissue sample is being fixed in a solution of formalin (also known as formaldehyde). Chemical

fixation kills microorganisms in the specimen, stopping degradation of the tissues and preserving their structure so that they can be examined later under the microscope. (credit a: modification of work by Nina Parker; credit b: modification of work by Nina Parker; credit c: modification of work by “University of Bristol”/YouTube)

In addition to fixation, **staining** is almost always applied to color certain features of a specimen before examining it under a light microscope. Stains, or dyes, contain salts made up of a positive ion and a negative ion. Depending on the type of dye, the positive or the negative ion may be the chromophore (the colored ion); the other, uncolored ion is called the counterion. If the chromophore is the positively charged ion, the stain is classified as a **basic dye**; if the negative ion is the chromophore, the stain is considered an **acidic dye**.

Dyes are selected for staining based on the chemical properties of the dye and the specimen being observed, which determine how the dye will interact with the specimen. In most cases, it is preferable to use a **positive stain**, a dye that will be absorbed by the cells or organisms being observed, adding color to objects of interest to make them stand out against the background. However, there are scenarios in which it is advantageous to use a **negative stain**, which is absorbed by the background but not by the cells or organisms in the specimen. Negative staining produces an outline or silhouette of the organisms against a colorful background ([\[link\]](#)).



(a)



(b)



(c)

(a) These *Bacillus anthracis* cells have absorbed crystal violet, a basic positive stain. (b) This specimen of *Spinoloricus*, a microscopic marine organism, has been stained with rose bengal, a positive acidic stain. (c) These *B. megaterium* appear to be white because they have not absorbed the negative red stain applied to the slide. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by Roberto Danovaro, Antonio Pusceddu, Cristina Gambi, Iben Heiner, Reinhardt Mobjerg Kristensen; credit c: modification of work by Anh-Hue Tu)

Because cells typically have negatively charged cell walls, the positive chromophores in basic dyes tend to stick to the cell walls, making them positive stains. Thus, commonly used basic dyes such as basic fuchsin, crystal violet, malachite green, methylene blue, and safranin typically serve as positive stains. On the other hand, the negatively charged chromophores in acidic dyes are repelled by negatively charged cell walls, making them negative stains. Commonly used acidic dyes include acid fuchsin, eosin, and rose bengal. [\[link\]](#) provides more detail.

Some staining techniques involve the application of only one dye to the sample; others require more than one dye. In **simple staining**, a single dye is used to emphasize particular structures in the specimen. A simple stain will generally make all of the organisms in a sample appear to be the same color, even if the sample contains more than one type of organism. In contrast, **differential staining** distinguishes organisms based on their interactions with multiple stains. In other words, two organisms in a differentially stained sample may appear to be different colors. Differential staining techniques commonly used in clinical settings include Gram staining, acid-fast staining, endospore staining, flagella staining, and capsule staining. [\[link\]](#) provides more detail on these differential staining techniques.

Note:

- Explain why it is important to fix a specimen before viewing it under a light microscope.
- What types of specimens should be chemically fixed as opposed to heat-fixed?
- Why might an acidic dye react differently with a given specimen than a basic dye?
- Explain the difference between a positive stain and a negative stain.
- Explain the difference between simple and differential staining.

Gram Staining

The **Gram stain procedure** is a differential staining procedure that involves multiple steps. It was developed by Danish microbiologist Hans Christian Gram in 1884 as an effective method to distinguish between bacteria with different types of cell walls, and even today it remains one of the most frequently used staining techniques. The steps of the Gram stain procedure are listed below and illustrated in [\[link\]](#).

1. First, crystal violet, a **primary stain**, is applied to a heat-fixed smear, giving all of the cells a purple color.
2. Next, Gram's iodine, a **mordant**, is added. A mordant is a substance used to set or stabilize stains or dyes; in this case, Gram's iodine acts like a trapping agent that complexes with the crystal violet, making the crystal violet–iodine complex clump and stay contained in thick layers of peptidoglycan in the cell walls.
3. Next, a **decolorizing agent** is added, usually ethanol or an acetone/ethanol solution. Cells that have thick peptidoglycan layers in their cell walls are much less affected by the decolorizing agent; they generally retain the crystal violet dye and remain purple. However, the decolorizing agent more easily washes the dye out of cells with thinner peptidoglycan layers, making them again colorless.
4. Finally, a secondary **counterstain**, usually safranin, is added. This stains the decolorized cells pink and is less noticeable in the cells that still contain the crystal violet dye.

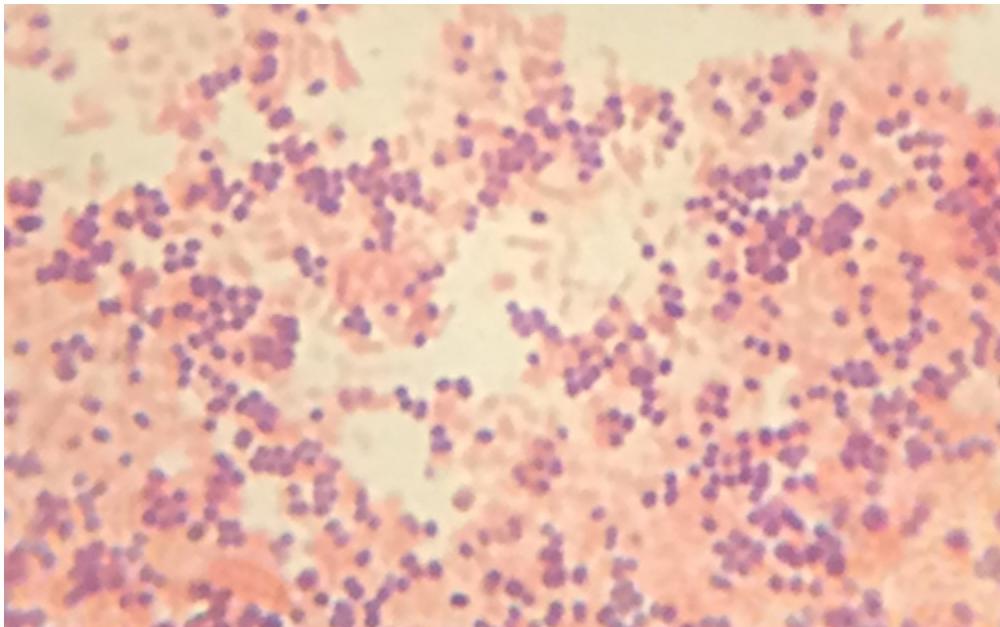
Gram stain process			
Gram staining steps	Cell effects	Gram-positive	Gram-negative
Step 1 Crystal violet <i>primary stain added to specimen smear.</i>	Stains cells purple or blue.		
Step 2 Iodine <i>mordant makes dye less soluble so it adheres to cell walls.</i>	Cells remain purple or blue.		
Step 3 Alcohol <i>decolorizer washes away stain from gram-negative cell walls.</i>	Gram-positive cells remain purple or blue. Gram-negative cells are colorless.		
Step 4 Safranin <i>counterstain allows dye adherence to gram-negative cells.</i>	Gram-positive cells remain purple or blue. Gram-negative cells appear pink or red.		

Gram-staining is a differential staining technique that uses a primary stain and a secondary counterstain to distinguish between gram-positive and gram-negative bacteria.

The purple, crystal-violet stained cells are referred to as gram-positive cells, while the red, safranin-dyed cells are gram-negative ([\[link\]](#)). However, there are several important considerations in interpreting the results of a Gram stain. First, older bacterial cells may have damage to their cell walls that causes them to appear gram-negative even if the species is gram-positive. Thus, it is best to use fresh bacterial cultures for Gram staining. Second, errors such as leaving on decolorizer too long can affect the results. In some cases, most cells will appear gram-positive while a few appear gram-negative (as in [\[link\]](#)). This suggests damage to the individual cells or

that decolorizer was left on for too long; the cells should still be classified as gram-positive if they are all the same species rather than a mixed culture.

Besides their differing interactions with dyes and decolorizing agents, the chemical differences between gram-positive and gram-negative cells have other implications with clinical relevance. For example, Gram staining can help clinicians classify bacterial pathogens in a sample into categories associated with specific properties. Gram-negative bacteria tend to be more resistant to certain antibiotics than gram-positive bacteria. We will discuss this and other applications of Gram staining in more detail in later chapters.



In this specimen, the gram-positive bacterium *Staphylococcus aureus* retains crystal violet dye even after the decolorizing agent is added. Gram-negative *Escherichia coli*, the most common Gram stain quality-control bacterium, is decolorized, and is only visible after the addition of the pink counterstain safranin.
(credit: modification of work by Nina Parker)

Note:

- Explain the role of Gram's iodine in the Gram stain procedure.
- Explain the role of alcohol in the Gram stain procedure.
- What color are gram-positive and gram-negative cells, respectively, after the Gram stain procedure?

Acid-Fast Stains

Acid-fast staining is another commonly used, differential staining technique that can be an important diagnostic tool. An **acid-fast stain** is able to differentiate two types of gram-positive cells: those that have waxy mycolic acids in their cell walls, and those that do not. Two different methods for acid-fast staining are the **Ziehl-Neelsen technique** and the **Kinyoun technique**. Both use carbolfuchsin as the primary stain. The waxy, acid-fast cells retain the carbolfuchsin even after a decolorizing agent (an acid-alcohol solution) is applied. A secondary counterstain, methylene blue, is then applied, which renders non-acid-fast cells blue.

The fundamental difference between the two carbolfuchsin-based methods is whether heat is used during the primary staining process. The Ziehl-Neelsen method uses heat to infuse the carbolfuchsin into the acid-fast cells, whereas the Kinyoun method does not use heat. Both techniques are important diagnostic tools because a number of specific diseases are caused by acid-fast bacteria (AFB). If AFB are present in a tissue sample, their red or pink color can be seen clearly against the blue background of the surrounding tissue cells ([\[link\]](#)).

Note:

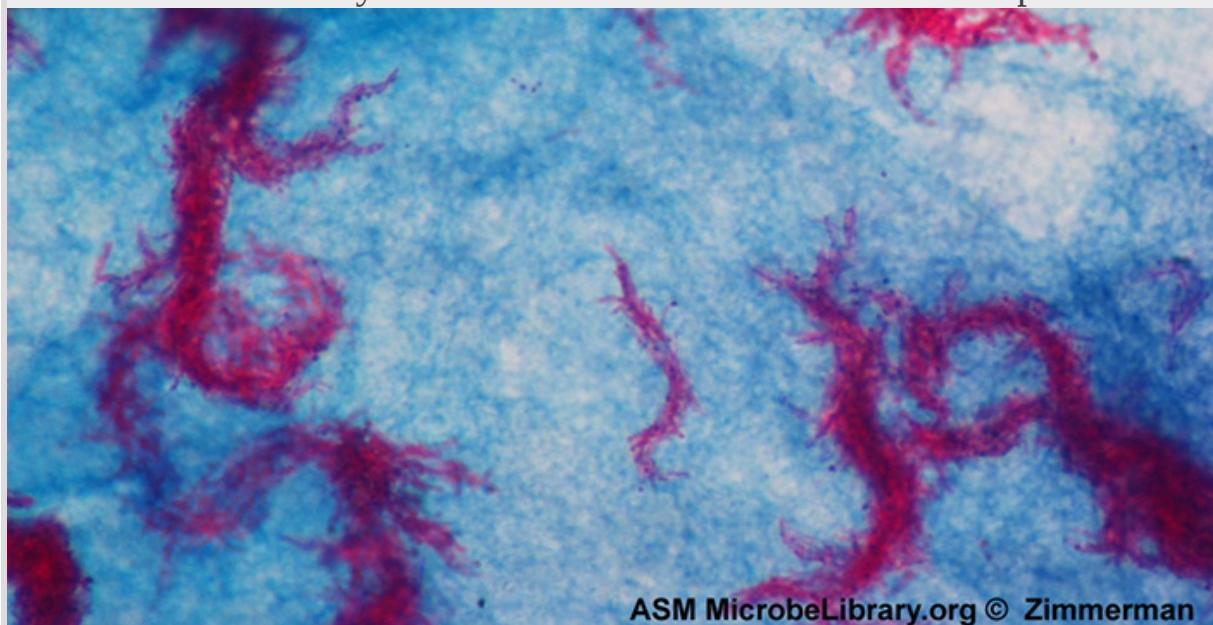
- Why are acid-fast stains useful?

Note:

Using Microscopy to Diagnose Tuberculosis

Mycobacterium tuberculosis, the bacterium that causes tuberculosis, can be detected in specimens based on the presence of acid-fast bacilli. Often, a smear is prepared from a sample of the patient's sputum and then stained using the Ziehl-Neelsen technique ([\[link\]](#)). If acid-fast bacteria are confirmed, they are generally cultured to make a positive identification. Variations of this approach can be used as a first step in determining whether *M. tuberculosis* or other acid-fast bacteria are present, though samples from elsewhere in the body (such as urine) may contain other *Mycobacterium* species.

An alternative approach for determining the presence of *M. tuberculosis* is immunofluorescence. In this technique, fluorochrome-labeled antibodies bind to *M. tuberculosis*, if present. Antibody-specific fluorescent dyes can be used to view the mycobacteria with a fluorescence microscope.



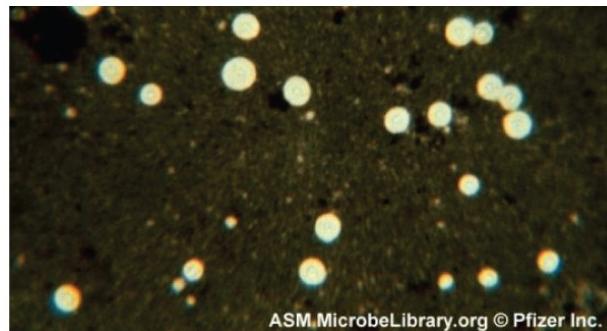
ASM MicrobeLibrary.org © Zimmerman

Ziehl-Neelsen staining has rendered these *Mycobacterium tuberculosis* cells red and the surrounding growth indicator medium blue. (credit: modification of work by American Society for Microbiology)

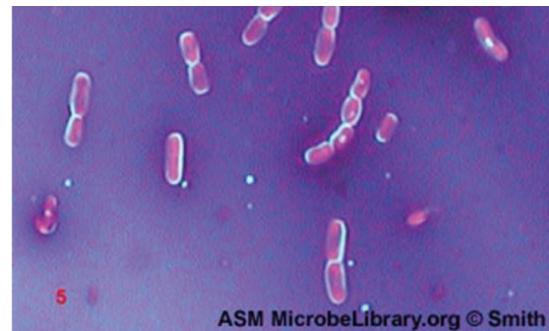
Capsule Staining

Certain bacteria and yeasts have a protective outer structure called a capsule. Since the presence of a capsule is directly related to a microbe's virulence (its ability to cause disease), the ability to determine whether cells in a sample have capsules is an important diagnostic tool. Capsules do not absorb most basic dyes; therefore, a negative staining technique (staining around the cells) is typically used for **capsule staining**. The dye stains the background but does not penetrate the capsules, which appear like halos around the borders of the cell. The specimen does not need to be heat-fixed prior to negative staining.

One common negative staining technique for identifying encapsulated yeast and bacteria is to add a few drops of India ink or nigrosin to a specimen. Other capsular stains can also be used to negatively stain encapsulated cells ([\[link\]](#)). Alternatively, positive and negative staining techniques can be combined to visualize capsules: The positive stain colors the body of the cell, and the negative stain colors the background but not the capsule, leaving halo around each cell.



(a)



(b)

- (a) India-ink was used to stain the background around these cells of the yeast *Cryptococcus neoformans*. The halos surrounding the cells are the polysaccharide capsules. (b) Crystal violet and copper sulfate dyes cannot penetrate the encapsulated *Bacillus* cells in this negatively stained sample. Encapsulated cells appear to have a light-blue halo.

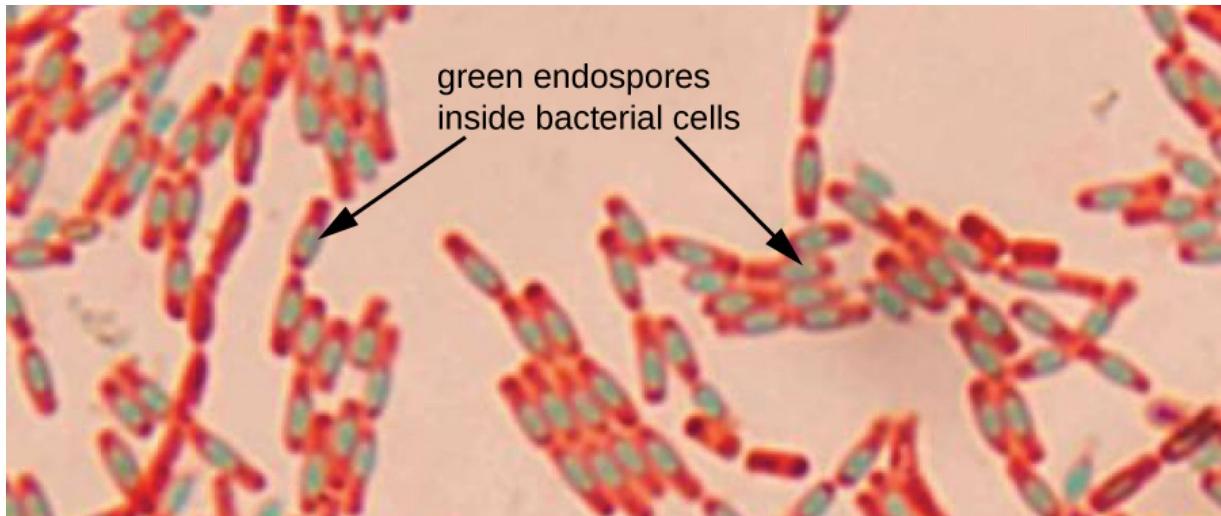
(credit a: modification of work by American Society for Microbiology;
credit b: modification of work by American Society for Microbiology)

Note:

- How does negative staining help us visualize capsules?

Endospore Staining

Endospores are structures produced within certain bacterial cells that allow them to survive harsh conditions. Gram staining alone cannot be used to visualize endospores, which appear clear when Gram-stained cells are viewed. **Endospore staining** uses two stains to differentiate endospores from the rest of the cell. The Schaeffer-Fulton method (the most commonly used endospore-staining technique) uses heat to push the primary stain (malachite green) into the endospore. Washing with water decolorizes the cell, but the endospore retains the green stain. The cell is then counterstained pink with safranin. The resulting image reveals the shape and location of endospores, if they are present. The green endospores will appear either within the pink vegetative cells or as separate from the pink cells altogether. If no endospores are present, then only the pink vegetative cells will be visible ([\[link\]](#)).



A stained preparation of *Bacillus subtilis* showing endospores as green and the vegetative cells as pink. (credit: modification of work by American Society for Microbiology)

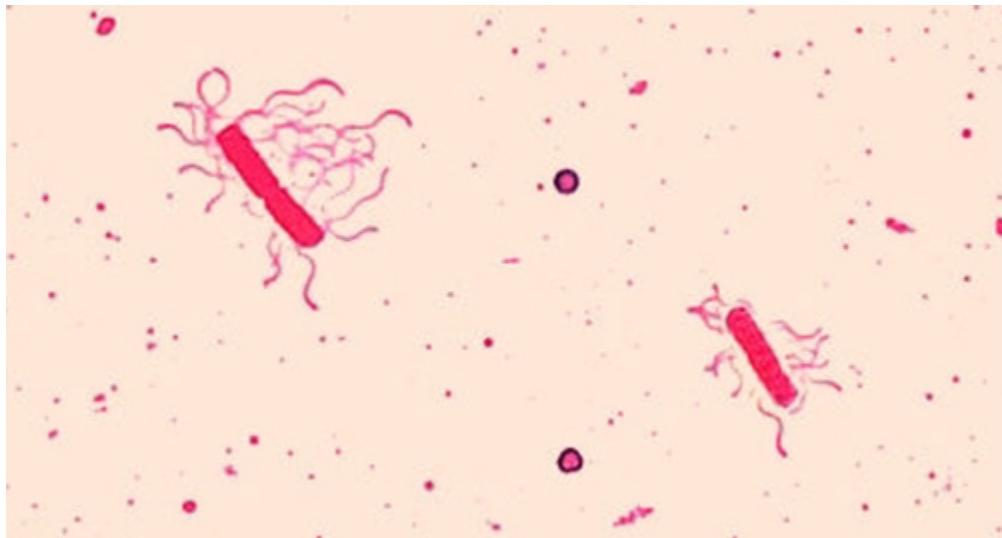
Endospore-staining techniques are important for identifying *Bacillus* and *Clostridium*, two genera of endospore-producing bacteria that contain clinically significant species. Among others, *B. anthracis* (which causes anthrax) has been of particular interest because of concern that its spores could be used as a bioterrorism agent. *C. difficile* is a particularly important species responsible for the typically hospital-acquired infection known as “C. diff.”

Note:

- Is endospore staining an example of positive, negative, or differential staining?

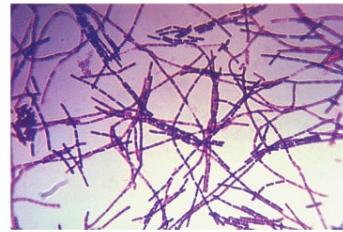
Flagella Staining

Flagella (singular: flagellum) are tail-like cellular structures used for locomotion by some bacteria, archaea, and eukaryotes. Because they are so thin, flagella typically cannot be seen under a light microscope without a specialized **flagella staining** technique. Flagella staining thickens the flagella by first applying mordant (generally tannic acid, but sometimes potassium alum), which coats the flagella; then the specimen is stained with pararosaniline (most commonly) or basic fuchsin ([\[link\]](#)).

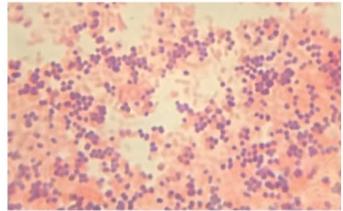
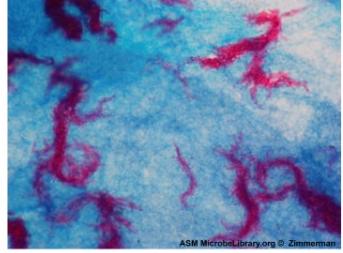
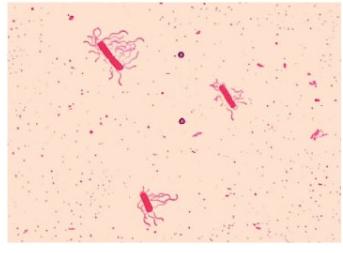
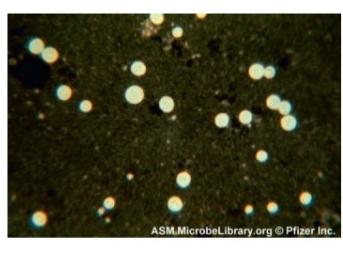


A flagella stain of *Bacillus cereus*, a common cause of foodborne illness, reveals that the cells have numerous flagella, used for locomotion. (credit: modification of work by Centers for Disease Control and Prevention)

Though flagella staining is uncommon in clinical settings, the technique is commonly used by microbiologists, since the location and number of flagella can be useful in classifying and identifying bacteria in a sample. When using this technique, it is important to handle the specimen with great care; flagella are delicate structures that can easily be damaged or pulled off, compromising attempts to accurately locate and count the number of flagella.

SIMPLE STAINS				
Stain Type	Specific Dyes	Purpose	Outcome	Sample Images
Basic stains	Methylene blue, crystal violet, malachite green, basic fuchsin, carbolfuchsin, safranin	Stain negatively charged molecules and structures, such as nucleic acids and proteins	Positive stain	
Acidic stains	Eosin, acid fuchsin, rose bengal, Congo red	Stain positively charged molecules and structures, such as proteins	Can be either a positive or negative stain, depending on the cell's chemistry.	
Negative stains	India ink, nigrosin	Stains background, not specimen	Dark background with light specimen	

(credit “basic stains”: modification of work by Centers for Disease Control and Prevention; credit “Acidic stains”: modification of work by Roberto Danovaro, Antonio Dell’Anno, Antonio Pusceddu, Cristina Gambi, Iben Heiner, Reinhhardt Mobjerg Kristensen; credit “Negative stains”: modification of work by Anh-Hue Tu)

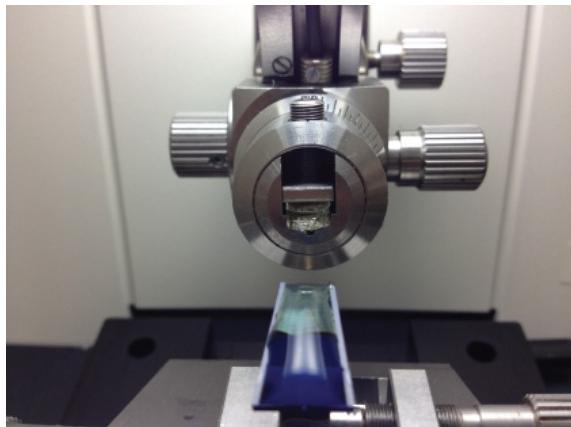
DIFFERENTIAL STAINS				
Stain Type	Specific Dyes	Purpose	Outcome	Sample Images
Gram stain	Uses crystal violet, Gram's iodine, ethanol (decolorizer), and safranin	Used to distinguish cells by cell-wall type (gram-positive, gram-negative)	Gram-positive cells stain purple/violet. Gram-negative cells stain pink.	
Acid-fast stain	After staining with basic fuchsin, acid-fast bacteria resist decolorization by acid-alcohol. Non acid-fast bacteria are counterstained with methylene blue.	Used to distinguish acid-fast bacteria such as <i>M. tuberculosis</i> , from non-acid-fast cells	Acid-fast bacteria are red; non-acid-fast cells are blue.	 ASM MicrobeLibrary.org © Zimmerman
Endospore stain	Uses heat to stain endospores with malachite green (Schaeffer-Fulton procedure), then cell is washed and counterstained with safranin.	Used to distinguish organisms with endospores from those without; used to study the endospore.	Endospores appear bluish-green; other structures appear pink to red.	
Flagella stain	Flagella are coated with a tannic acid or potassium alum mordant, then stained using either pararosaline or basic fuchsin.	Used to view and study flagella in bacteria that have them.	Flagella are visible if present.	
Capsule stain	Negative staining with India ink or nigrosin is used to stain the background, leaving a clear area of the cell and the capsule. Counterstaining can be used to stain the cell while leaving the capsule clear.	Used to distinguish cells with capsules from those without.	Capsules appear clear or as halos if present.	 ASM MicrobeLibrary.org © Pfizer Inc.

(credit “Gram stain”: modification of work by Nina Parker; credit “Acid-fast stain”: modification of work by American Society for Microbiology; credit “Endospore stain”: modification of work by

American Society for Microbiology; credit “Capsule stain” : modification of work by American Society for Microbiology; credit “Flagella stain”: modification of work by Centers for Disease Control and Prevention)

Preparing Specimens for Electron Microscopy

Samples to be analyzed using a TEM must have very thin sections. But cells are too soft to cut thinly, even with diamond knives. To cut cells without damage, the cells must be embedded in plastic resin and then dehydrated through a series of soaks in ethanol solutions (50%, 60%, 70%, and so on). The ethanol replaces the water in the cells, and the resin dissolves in ethanol and enters the cell, where it solidifies. Next, **thin sections** are cut using a specialized device called an **ultramicrotome** ([\[link\]](#)). Finally, samples are fixed to fine copper wire or carbon-fiber grids and stained—not with colored dyes, but with substances like uranyl acetate or osmium tetroxide, which contain electron-dense heavy metal atoms.



(a)



(b)

(a) An ultramicrotome used to prepare specimens for a TEM. (b) A technician uses an ultramicrotome to slice a specimen into thin sections. (credit a: modification of work by “Frost Museum”/Flickr;

credit b: modification of work by U.S. Fish and Wildlife Service
Northeast Region)

When samples are prepared for viewing using an SEM, they must also be dehydrated using an ethanol series. However, they must be even drier than is necessary for a TEM. Critical point drying with inert liquid carbon dioxide under pressure is used to displace the water from the specimen. After drying, the specimens are sputter-coated with metal by knocking atoms off of a palladium target, with energetic particles. Sputter-coating prevents specimens from becoming charged by the SEM's electron beam.

Note:

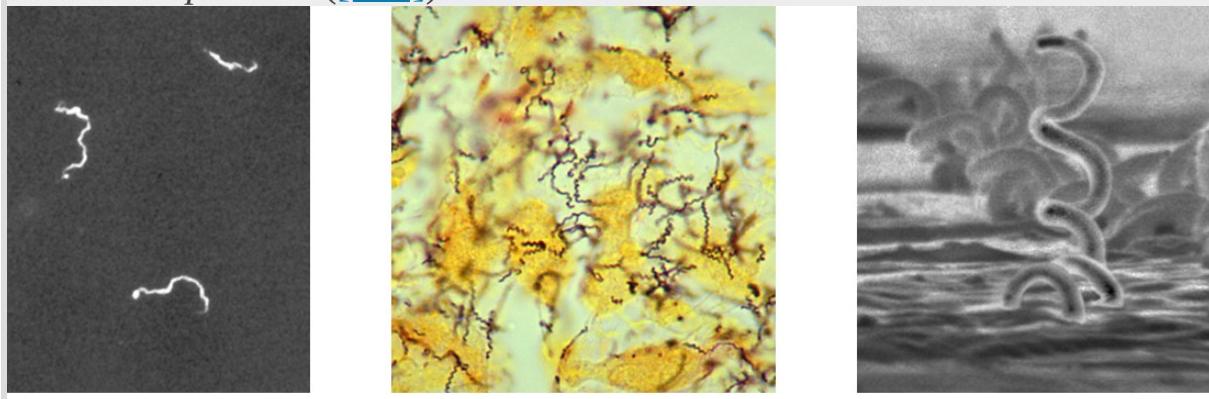
- Why is it important to dehydrate cells before examining them under an electron microscope?
- Name the device that is used to create thin sections of specimens for electron microscopy.

Note:

Using Microscopy to Diagnose Syphilis

The causative agent of syphilis is *Treponema pallidum*, a flexible, spiral cell (spirochete) that can be very thin (<0.15 μm) and match the refractive index of the medium, making it difficult to view using brightfield microscopy. Additionally, this species has not been successfully cultured in the laboratory on an artificial medium; therefore, diagnosis depends upon successful identification using microscopic techniques and serology (analysis of body fluids, often looking for antibodies to a pathogen). Since fixation and staining would kill the cells, darkfield microscopy is typically used for observing live specimens and viewing their movements. However, other approaches can also be used. For example, the cells can be thickened with silver particles (in tissue sections) and observed using a light

microscope. It is also possible to use fluorescence or electron microscopy to view *Treponema* ([\[link\]](#)).



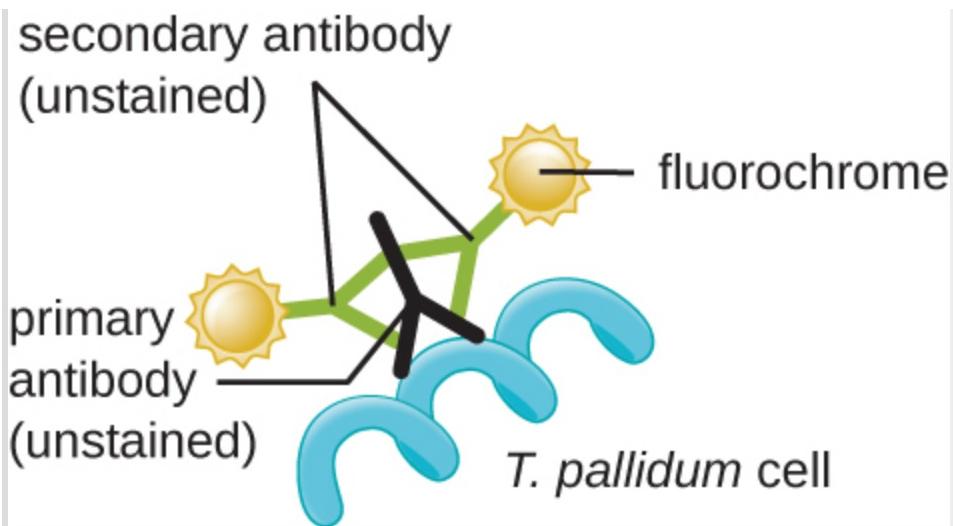
(a)

(b)

(c)

(a) Living, unstained *Treponema pallidum* spirochetes can be viewed under a darkfield microscope. (b) In this brightfield image, a modified Steiner silver stain is used to visualize *T. pallidum* spirochetes. Though the stain kills the cells, it increases the contrast to make them more visible. (c) While not used for standard diagnostic testing, *T. pallidum* can also be examined using scanning electron microscopy.
(credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by Centers for Disease Control and Prevention; credit c: modification of work by Centers for Disease Control and Prevention)

In clinical settings, indirect immunofluorescence is often used to identify *Treponema*. A primary, unstained antibody attaches directly to the pathogen surface, and secondary antibodies “tagged” with a fluorescent stain attach to the primary antibody. Multiple secondary antibodies can attach to each primary antibody, amplifying the amount of stain attached to each *Treponema* cell, making them easier to spot ([\[link\]](#)).



Indirect immunofluorescence can be used to identify *T. pallidum*, the causative agent of syphilis, in a specimen.

Preparation and Staining for Other Microscopes

Samples for fluorescence and confocal microscopy are prepared similarly to samples for light microscopy, except that the dyes are fluorochromes. Stains are often diluted in liquid before applying to the slide. Some dyes attach to an antibody to stain specific proteins on specific types of cells (immunofluorescence); others may attach to DNA molecules in a process called fluorescence in situ hybridization (FISH), causing cells to be stained based on whether they have a specific DNA sequence.

Sample preparation for two-photon microscopy is similar to fluorescence microscopy, except for the use of infrared dyes. Specimens for STM need to be on a very clean and atomically smooth surface. They are often mica coated with Au(111). Toluene vapor is a common fixative.

Note:

- What is the main difference between preparing a sample for fluorescence microscopy versus light microscopy?

Note:

Cornell University's [Case Studies in Microscopy](#) offers a series of clinical problems based on real-life events. Each case study walks you through a clinical problem using appropriate techniques in microscopy at each step.

Note:**Resolution**

From the results of the Gram stain, the technician now knows that Cindy's infection is caused by spherical, gram-positive bacteria that form grape-like clusters, which is typical of staphylococcal bacteria. After some additional testing, the technician determines that these bacteria are the medically important species known as *Staphylococcus aureus*, a common culprit in wound infections. Because some strains of *S. aureus* are resistant to many antibiotics, skin infections may spread to other areas of the body and become serious, sometimes even resulting in amputations or death if the correct antibiotics are not used.

After testing several antibiotics, the lab is able to identify one that is effective against this particular strain of *S. aureus*. Cindy's doctor quickly prescribes the medication and emphasizes the importance of taking the

entire course of antibiotics, even if the infection appears to clear up before the last scheduled dose. This reduces the risk that any especially resistant bacteria could survive, causing a second infection or spreading to another person.

Go back to the [previous](#) Clinical Focus box.

Note:

Microscopy and Antibiotic Resistance

As the use of antibiotics has proliferated in medicine, as well as agriculture, microbes have evolved to become more resistant. Strains of bacteria such as methicillin-resistant *S. aureus* (MRSA), which has developed a high level of resistance to many antibiotics, are an increasingly worrying problem, so much so that research is underway to develop new and more diversified antibiotics.

Fluorescence microscopy can be useful in testing the effectiveness of new antibiotics against resistant strains like MRSA. In a test of one new antibiotic derived from a marine bacterium, MC21-A (bromophene), researchers used the fluorescent dye SYTOX Green to stain samples of MRSA. SYTOX Green is often used to distinguish dead cells from living cells, with fluorescence microscopy. Live cells will not absorb the dye, but cells killed by an antibiotic will absorb the dye, since the antibiotic has damaged the bacterial cell membrane. In this particular case, MRSA bacteria that had been exposed to MC21-A did, indeed, appear green under the fluorescence microscope, leading researchers to conclude that it is an effective antibiotic against MRSA.

Of course, some argue that developing new antibiotics will only lead to even more antibiotic-resistant microbes, so-called superbugs that could spawn epidemics before new treatments can be developed. For this reason, many health professionals are beginning to exercise more discretion in prescribing antibiotics. Whereas antibiotics were once routinely prescribed for common illnesses without a definite diagnosis, doctors and hospitals are much more likely to conduct additional testing to determine whether an antibiotic is necessary and appropriate before prescribing.

A sick patient might reasonably object to this stingy approach to prescribing antibiotics. To the patient who simply wants to feel better as

quickly as possible, the potential benefits of taking an antibiotic may seem to outweigh any immediate health risks that might occur if the antibiotic is ineffective. But at what point do the risks of widespread antibiotic use supersede the desire to use them in individual cases?

Key Concepts and Summary

- Samples must be properly prepared for microscopy. This may involve **staining, fixation**, and/or cutting **thin sections**.
- A variety of staining techniques can be used with light microscopy, including **Gram staining, acid-fast staining, capsule staining, endospore staining, and flagella staining**.
- Samples for TEM require very thin sections, whereas samples for SEM require sputter-coating.
- Preparation for fluorescence microscopy is similar to that for light microscopy, except that fluorochromes are used.

Short Answer

Exercise:

Problem:

How could you identify whether a particular bacterial sample contained specimens with mycolic acid-rich cell walls?

Critical Thinking

Exercise:

Problem:

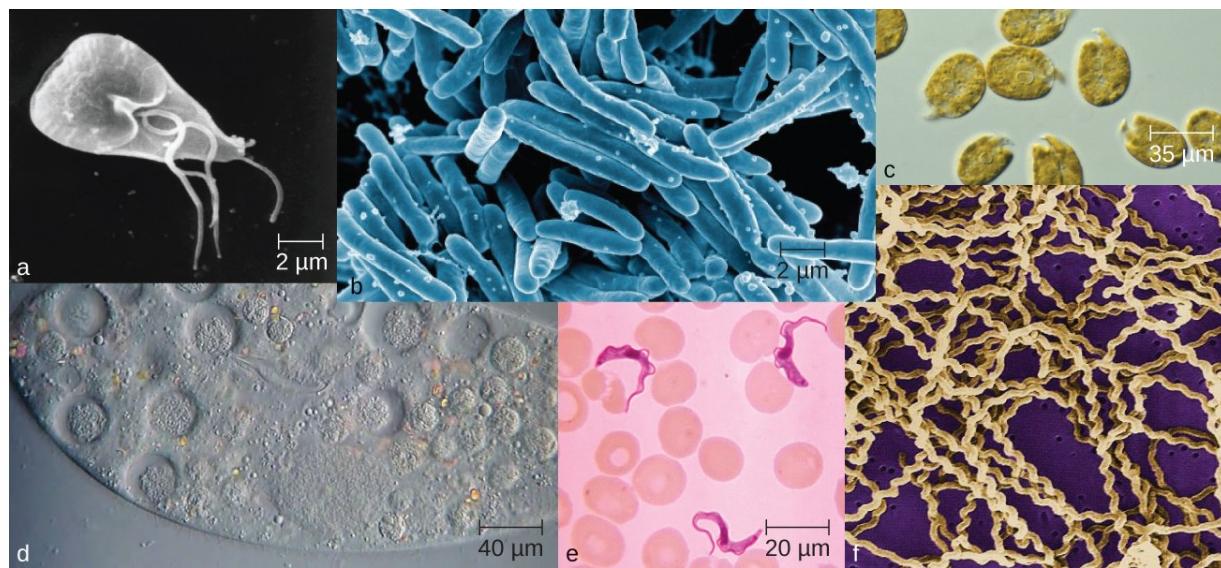
You use the Gram staining procedure to stain an L-form bacterium (a bacterium that lacks a cell wall). What color will the bacterium be after the staining procedure is finished?

The Cell - Introduction

class="introduction"

Microorganisms vary visually in their size and shape, as can be observed microscopically; but they also vary in invisible ways, such as in their metabolic capabilities.

(credit a, e, f: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by NIAID; credit c: modification of work by CSIRO; credit d: modification of work by “Microscopic World”/YouTube)



Life takes many forms, from giant redwood trees towering hundreds of feet in the air to the tiniest known microbes, which measure only a few billionths of a meter. Humans have long pondered life's origins and debated the defining characteristics of life, but our understanding of these concepts has changed radically since the invention of the microscope. In the 17th century, observations of microscopic life led to the development of the cell theory: the idea that the fundamental unit of life is the cell, that all organisms contain at least one cell, and that cells only come from other cells.

Despite sharing certain characteristics, cells may vary significantly. The two main types of cells are prokaryotic cells (lacking a nucleus) and eukaryotic cells (containing a well-organized, membrane-bound nucleus). Each type of cell exhibits remarkable variety in structure, function, and metabolic activity ([\[link\]](#)). This chapter will focus on the historical discoveries that have shaped our current understanding of microbes, including their origins and their role in human disease. We will then explore the distinguishing structures found in prokaryotic and eukaryotic cells.

Spontaneous Generation

LEARNING OBJECTIVES

- Explain the theory of spontaneous generation and why people once accepted it as an explanation for the existence of certain types of organisms
- Explain how certain individuals (van Helmont, Redi, Needham, Spallanzani, and Pasteur) tried to prove or disprove spontaneous generation
- Explain Pasteur experiment

Humans have been asking for millennia: Where does new life come from? Religion, philosophy, and science have all wrestled with this question. One of the oldest explanations was the theory of spontaneous generation, which can be traced back to the ancient Greeks and was widely accepted through the Middle Ages.

The Theory of Spontaneous Generation

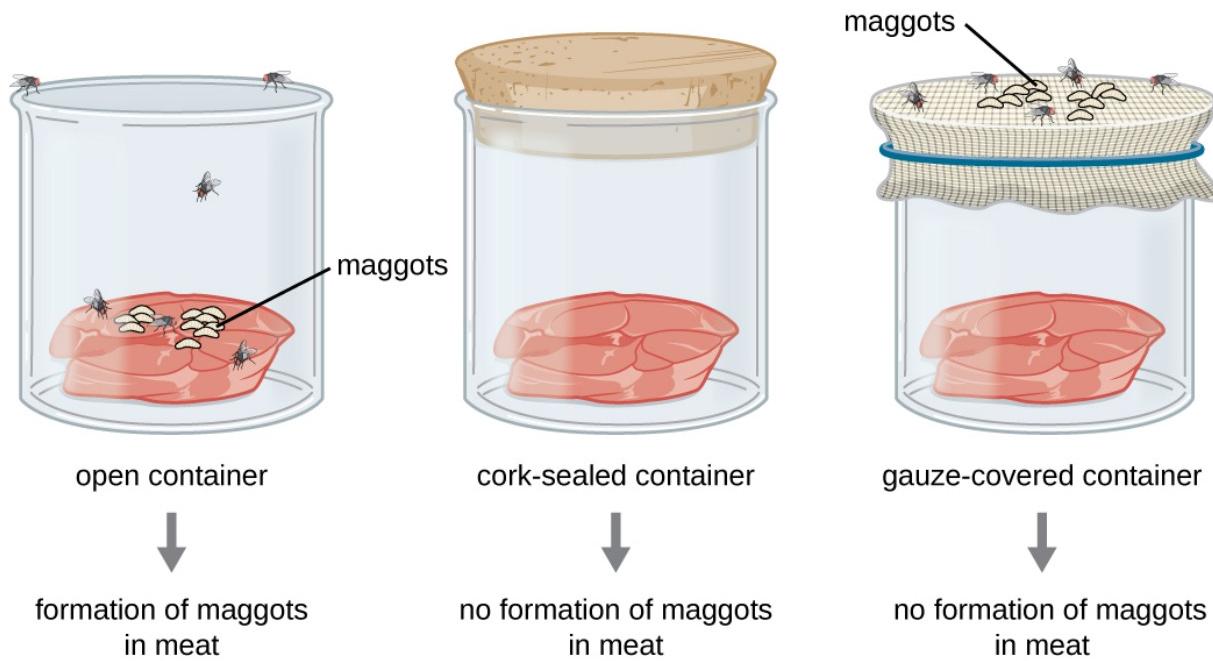
The Greek philosopher Aristotle (384–322 BC) was one of the earliest recorded scholars to articulate the theory of **spontaneous generation**, the notion that life can arise from nonliving matter. Aristotle proposed that life arose from nonliving material if the material contained *pneuma* (“vital heat”). As evidence, he noted several instances of the appearance of animals from environments previously devoid of such animals, such as the seemingly sudden appearance of fish in a new puddle of water.[\[footnote\]](#) K. Zwier. “Aristotle on Spontaneous Generation.”

<http://www.sju.edu/int/academics/cas/resources/gppc/pdf/Karen%20R.%20>

Zwier.pdf

This theory persisted into the 17th century, when scientists undertook additional experimentation to support or disprove it. By this time, the proponents of the theory cited how frogs simply seem to appear along the muddy banks of the Nile River in Egypt during the annual flooding. Others observed that mice simply appeared among grain stored in barns with thatched roofs. When the roof leaked and the grain molded, mice appeared. Jan Baptista van Helmont, a 17th century Flemish scientist, proposed that mice could arise from rags and wheat kernels left in an open container for 3 weeks. In reality, such habitats provided ideal food sources and shelter for mouse populations to flourish.

However, one of van Helmont's contemporaries, Italian physician Francesco Redi (1626–1697), performed an experiment in 1668 that was one of the first to refute the idea that maggots (the larvae of flies) spontaneously generate on meat left out in the open air. He predicted that preventing flies from having direct contact with the meat would also prevent the appearance of maggots. Redi left meat in each of six containers ([\[link\]](#)). Two were open to the air, two were covered with gauze, and two were tightly sealed. His hypothesis was supported when maggots developed in the uncovered jars, but no maggots appeared in either the gauze-covered or the tightly sealed jars. He concluded that maggots could only form when flies were allowed to lay eggs in the meat, and that the maggots were the offspring of flies, not the product of spontaneous generation.



Francesco Redi's experimental setup consisted of an open container, a container sealed with a cork top, and a container covered in mesh that let in air but not flies. Maggots only appeared on the meat in the open container. However, maggots were also found on the gauze of the gauze-covered container.

In 1745, John Needham (1713–1781) published a report of his own experiments, in which he briefly boiled broth infused with plant or animal matter, hoping to kill all preexisting microbes.[\[footnote\]](#) He then sealed the flasks. After a few days, Needham observed that the broth had become cloudy and a single drop contained numerous microscopic creatures. He argued that the new microbes must have arisen spontaneously. In reality, however, he likely did not boil the broth enough to kill all preexisting microbes.

E. Capanna. “Lazzaro Spallanzani: At the Roots of Modern Biology.” *Journal of Experimental Zoology* 285 no. 3 (1999):178–196.

Lazzaro Spallanzani (1729–1799) did not agree with Needham’s conclusions, however, and performed hundreds of carefully executed experiments using heated broth.[\[footnote\]](#) As in Needham’s experiment,

broth in sealed jars and unsealed jars was infused with plant and animal matter. Spallanzani's results contradicted the findings of Needham: Heated but sealed flasks remained clear, without any signs of spontaneous growth, unless the flasks were subsequently opened to the air. This suggested that microbes were introduced into these flasks from the air. In response to Spallanzani's findings, Needham argued that life originates from a "life force" that was destroyed during Spallanzani's extended boiling. Any subsequent sealing of the flasks then prevented new life force from entering and causing spontaneous generation ([\[link\]](#)).

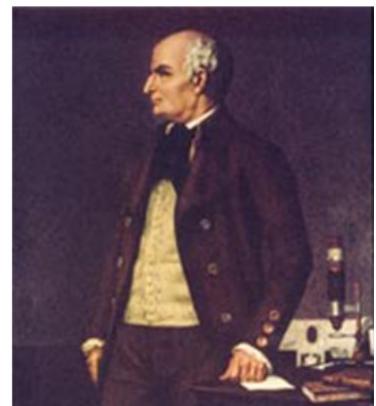
R. Mancini, M. Nigro, G. Ippolito. "Lazzaro Spallanzani and His Refutation of the Theory of Spontaneous Generation." *Le Infezioni in Medicina* 15 no. 3 (2007):199–206.



(a)



(b)



(c)

- (a) Francesco Redi, who demonstrated that maggots were the offspring of flies, not products of spontaneous generation. (b) John Needham, who argued that microbes arose spontaneously in broth from a "life force." (c) Lazzaro Spallanzani, whose experiments with broth aimed to disprove those of Needham.

Note:

- Describe the theory of spontaneous generation and some of the arguments used to support it.
- Explain how the experiments of Redi and Spallanzani challenged the theory of spontaneous generation.

Disproving Spontaneous Generation

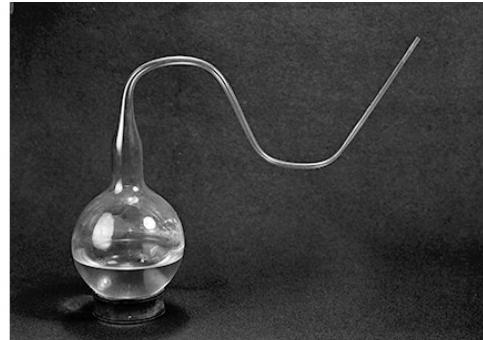
The debate over spontaneous generation continued well into the 19th century, with scientists serving as proponents of both sides. To settle the debate, the Paris Academy of Sciences offered a prize for resolution of the problem. Louis Pasteur, a prominent French chemist who had been studying microbial fermentation and the causes of wine spoilage, accepted the challenge. In 1858, Pasteur filtered air through a gun-cotton filter and, upon microscopic examination of the cotton, found it full of microorganisms, suggesting that the exposure of a broth to air was not introducing a “life force” to the broth but rather airborne microorganisms.

Later, Pasteur made a series of flasks with long, twisted necks (“swan-neck” flasks), in which he boiled broth to sterilize it ([\[link\]](#)). His design allowed air inside the flasks to be exchanged with air from the outside, but prevented the introduction of any airborne microorganisms, which would get caught in the twists and bends of the flasks’ necks. If a life force besides the airborne microorganisms were responsible for microbial growth within the sterilized flasks, it would have access to the broth, whereas the microorganisms would not. He correctly predicted that sterilized broth in his swan-neck flasks would remain sterile as long as the swan necks remained intact. However, should the necks be broken, microorganisms would be introduced, contaminating the flasks and allowing microbial growth within the broth.

Pasteur’s set of experiments irrefutably disproved the theory of spontaneous generation and earned him the prestigious Alhumbert Prize from the Paris Academy of Sciences in 1862. In a subsequent lecture in 1864, Pasteur articulated “*Omne vivum ex vivo*” (“Life only comes from life”). In this lecture, Pasteur recounted his famous swan-neck flask experiment, stating

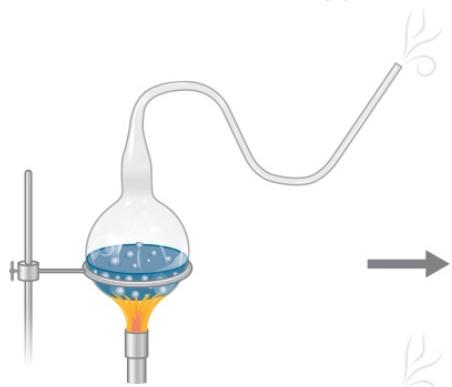
that "...life is a germ and a germ is life. Never will the doctrine of spontaneous generation recover from the mortal blow of this simple experiment."[\[footnote\]](#) To Pasteur's credit, it never has.

R. Vallery-Radot. *The Life of Pasteur*, trans. R.L. Devonshire. New York: McClure, Phillips and Co, 1902, 1:142.

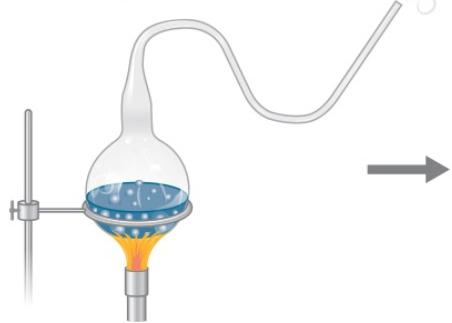


(a)

(b)



The curve of the flask prevents outside air from entering the flask. No contamination occurs.



When the neck of the flask is broken off, bacteria reach the sterile broth and organism growth occurs.



Boiling the broth kills microorganisms.

(c)

(a) French scientist Louis Pasteur, who definitively refuted the long-

disputed theory of spontaneous generation. (b) The unique swan-neck feature of the flasks used in Pasteur's experiment allowed air to enter the flask but prevented the entry of bacterial and fungal spores. (c) Pasteur's experiment consisted of two parts. In the first part, the broth in the flask was boiled to sterilize it. When this broth was cooled, it remained free of contamination. In the second part of the experiment, the flask was boiled and then the neck was broken off. The broth in this flask became contaminated. (credit b: modification of work by “Wellcome Images”/Wikimedia Commons)

Note:

- How did Pasteur's experimental design allow air, but not microbes, to enter, and why was this important?
- What was the control group in Pasteur's experiment and what did it show?

Key Concepts and Summary

- The theory of **spontaneous generation** states that life arose from nonliving matter. It was a long-held belief dating back to Aristotle and the ancient Greeks.
- Experimentation by Francesco Redi in the 17th century presented the first significant evidence refuting spontaneous generation by showing that flies must have access to meat for maggots to develop on the meat. Prominent scientists designed experiments and argued both in support of (John Needham) and against (Lazzaro Spallanzani) spontaneous generation.
- Louis Pasteur is credited with conclusively disproving the theory of spontaneous generation with his famous swan-neck flask experiment. He subsequently proposed that “life only comes from life.”

Critical Thinking

Exercise:

Problem:

What would the results of Pasteur's swan-neck flask experiment have looked like if they supported the theory of spontaneous generation?

Foundations of Modern Cell Theory

LEARNING OBJECTIVES

- Explain the key points of cell theory and the individual contributions of Hooke, Schleiden, Schwann, Remak, and Virchow
- Explain the key points of endosymbiotic theory and cite the evidence that supports this concept
- Explain the contributions of Semmelweis, Snow, Pasteur, Lister, and Koch to the development of germ theory

While some scientists were arguing over the theory of spontaneous generation, other scientists were making discoveries leading to a better understanding of what we now call the cell theory. Modern cell theory has two basic tenets:

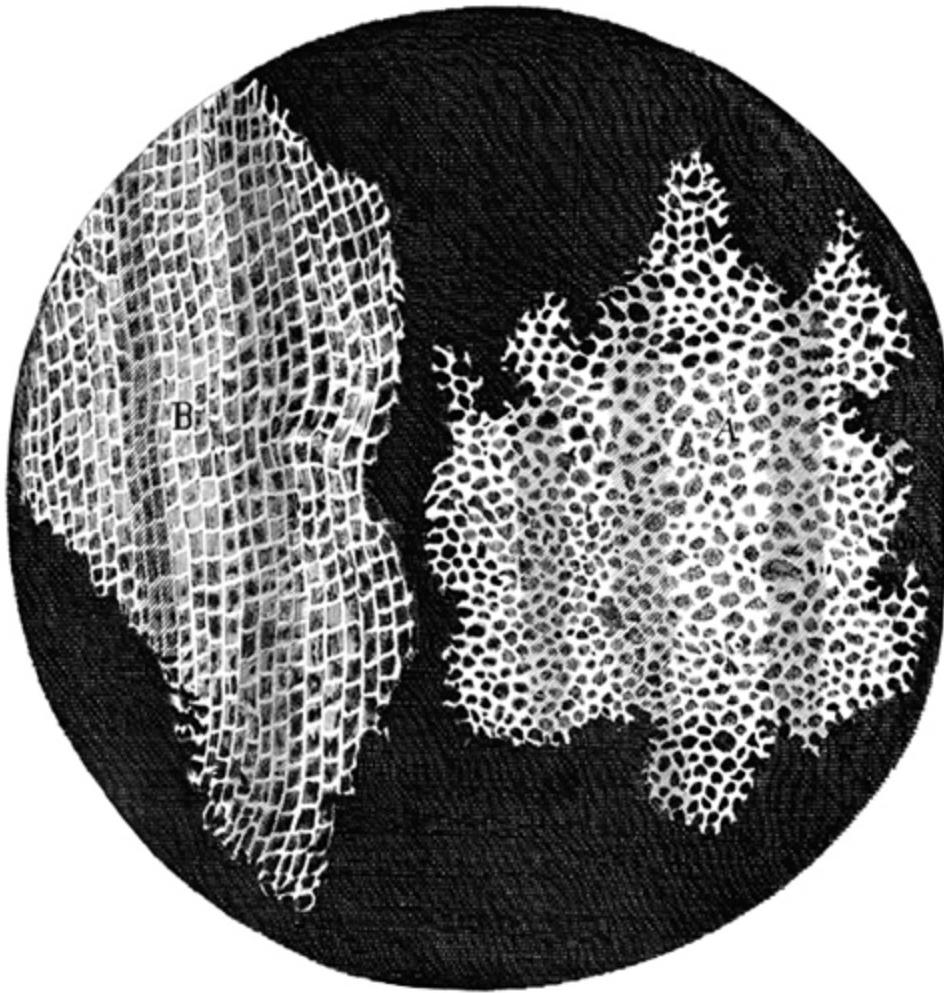
- All cells only come from other cells (the principle of biogenesis).
- Cells are the fundamental units of organisms.

Today, these tenets are fundamental to our understanding of life on earth. However, modern cell theory grew out of the collective work of many scientists.

The Origins of Cell Theory

The English scientist Robert Hooke first used the term “cells” in 1665 to describe the small chambers within cork that he observed under a microscope of his own design. To Hooke, thin sections of cork resembled

“Honey-comb,” or “small Boxes or Bladders of Air.” He noted that each “Cavern, Bubble, or Cell” was distinct from the others ([\[link\]](#)). At the time, Hooke was not aware that the cork cells were long dead and, therefore, lacked the internal structures found within living cells.



Robert Hooke (1635–1703) was the first to describe cells based upon his microscopic observations of cork. This illustration was published in his work *Micrographia*.

Despite Hooke’s early description of cells, their significance as the fundamental unit of life was not yet recognized. Nearly 200 years later, in

1838, Matthias Schleiden (1804–1881), a German botanist who made extensive microscopic observations of plant tissues, described them as being composed of cells. Visualizing plant cells was relatively easy because plant cells are clearly separated by their thick cell walls. Schleiden believed that cells formed through crystallization, rather than cell division.

Theodor Schwann (1810–1882), a noted German physiologist, made similar microscopic observations of animal tissue. In 1839, after a conversation with Schleiden, Schwann realized that similarities existed between plant and animal tissues. This laid the foundation for the idea that cells are the fundamental components of plants and animals.

In the 1850s, two Polish scientists living in Germany pushed this idea further, culminating in what we recognize today as the modern cell theory. In 1852, Robert Remak (1815–1865), a prominent neurologist and embryologist, published convincing evidence that cells are derived from other cells as a result of cell division. However, this idea was questioned by many in the scientific community. Three years later, Rudolf Virchow (1821–1902), a well-respected pathologist, published an editorial essay entitled “Cellular Pathology,” which popularized the concept of cell theory using the Latin phrase *omnis cellula a cellula* (“all cells arise from cells”), which is essentially the second tenet of modern cell theory.[\[footnote\]](#) Given the similarity of Virchow’s work to Remak’s, there is some controversy as to which scientist should receive credit for articulating cell theory. See the following Eye on Ethics feature for more about this controversy.

M. Schultz. “Rudolph Virchow.” *Emerging Infectious Diseases* 14 no. 9 (2008):1480–1481.

Note:

Science and Plagiarism

Rudolf Virchow, a prominent, Polish-born, German scientist, is often remembered as the “Father of Pathology.” Well known for innovative approaches, he was one of the first to determine the causes of various diseases by examining their effects on tissues and organs. He was also among the first to use animals in his research and, as a result of his work, he was the first to name numerous diseases and created many other

medical terms. Over the course of his career, he published more than 2,000 papers and headed various important medical facilities, including the Charité – Universitätsmedizin Berlin, a prominent Berlin hospital and medical school. But he is, perhaps, best remembered for his 1855 editorial essay titled “Cellular Pathology,” published in *Archiv für Pathologische Anatomie und Physiologie*, a journal that Virchow himself cofounded and still exists today.

Despite his significant scientific legacy, there is some controversy regarding this essay, in which Virchow proposed the central tenet of modern cell theory—that all cells arise from other cells. Robert Remak, a former colleague who worked in the same laboratory as Virchow at the University of Berlin, had published the same idea 3 years before. Though it appears Virchow was familiar with Remak’s work, he neglected to credit Remak’s ideas in his essay. When Remak wrote a letter to Virchow pointing out similarities between Virchow’s ideas and his own, Virchow was dismissive. In 1858, in the preface to one of his books, Virchow wrote that his 1855 publication was just an editorial piece, not a scientific paper, and thus there was no need to cite Remak’s work.

By today’s standards, Virchow’s editorial piece would certainly be considered an act of plagiarism, since he presented Remak’s ideas as his own. However, in the 19th century, standards for academic integrity were much less clear. Virchow’s strong reputation, coupled with the fact that Remak was a Jew in a somewhat anti-Semitic political climate, shielded him from any significant repercussions. Today, the process of peer review and the ease of access to the scientific literature help discourage plagiarism. Although scientists are still motivated to publish original ideas that advance scientific knowledge, those who would consider plagiarizing are well aware of the serious consequences.

In academia, plagiarism represents the theft of both individual thought and research—an offense that can destroy reputations and end careers.

[\[footnote\]](#) [\[footnote\]](#) [\[footnote\]](#) [\[footnote\]](#)

B. Kisch. “Forgotten Leaders in Modern Medicine, Valentin, Gouby, Remak, Auerbach.” *Transactions of the American Philosophical Society* 44 (1954):139–317.

H. Harris. *The Birth of the Cell*. New Haven, CT: Yale University Press, 2000:133.

C. Webster (ed.). *Biology, Medicine and Society 1840-1940*. Cambridge, UK; Cambridge University Press, 1981:118–119.

C. Zuchora-Walske. *Key Discoveries in Life Science*. Minneapolis, MN: Lerner Publishing, 2015:12–13.



(a)



(b)

(a) Rudolf Virchow (1821–1902) popularized the cell theory in an 1855 essay entitled “Cellular Pathology.” (b) The idea that all cells originate from other cells was first published in 1852 by his contemporary and former colleague Robert Remak (1815–1865).

Note:

- What are the key points of the cell theory?
- What contributions did Rudolf Virchow and Robert Remak make to the development of the cell theory?

Endosymbiotic Theory

As scientists were making progress toward understanding the role of cells in plant and animal tissues, others were examining the structures within the cells themselves. In 1831, Scottish botanist Robert Brown (1773–1858) was the first to describe observations of nuclei, which he observed in plant cells. Then, in the early 1880s, German botanist Andreas Schimper (1856–1901) was the first to describe the chloroplasts of plant cells, identifying their role in starch formation during photosynthesis and noting that they divided independent of the nucleus.

Based upon the chloroplasts' ability to reproduce independently, Russian botanist Konstantin Mereschkowski (1855–1921) suggested in 1905 that chloroplasts may have originated from ancestral photosynthetic bacteria living symbiotically inside a eukaryotic cell. He proposed a similar origin for the nucleus of plant cells. This was the first articulation of the endosymbiotic hypothesis, and would explain how eukaryotic cells evolved from ancestral bacteria.

Mereschkowski's endosymbiotic hypothesis was furthered by American anatomist Ivan Wallin (1883–1969), who began to experimentally examine the similarities between mitochondria, chloroplasts, and bacteria—in other words, to put the endosymbiotic hypothesis to the test using objective investigation. Wallin published a series of papers in the 1920s supporting the endosymbiotic hypothesis, including a 1926 publication co-authored with Mereschkowski. Wallin claimed he could culture mitochondria outside of their eukaryotic host cells. Many scientists dismissed his cultures of mitochondria as resulting from bacterial contamination. Modern genome sequencing work supports the dissenting scientists by showing that much of the genome of mitochondria had been transferred to the host cell's nucleus, preventing the mitochondria from being able to live on their own.[\[footnote\]](#) [\[footnote\]](#)

T. Embley, W. Martin. "Eukaryotic Evolution, Changes, and Challenges." *Nature* Vol. 440 (2006):623–630.

O.G. Berg, C.G. Kurland. "Why Mitochondrial Genes Are Most Often Found in Nuclei." *Molecular Biology and Evolution* 17 no. 6 (2000):951–961.

Wallin's ideas regarding the endosymbiotic hypothesis were largely ignored for the next 50 years because scientists were unaware that these organelles contained their own DNA. However, with the discovery of mitochondrial and chloroplast DNA in the 1960s, the endosymbiotic hypothesis was resurrected. Lynn Margulis (1938–2011), an American geneticist, published her ideas regarding the endosymbiotic hypothesis of the origins of mitochondria and chloroplasts in 1967.[\[footnote\]](#) In the decade leading up to her publication, advances in microscopy had allowed scientists to differentiate prokaryotic cells from eukaryotic cells. In her publication, Margulis reviewed the literature and argued that the eukaryotic organelles such as mitochondria and chloroplasts are of prokaryotic origin. She presented a growing body of microscopic, genetic, molecular biology, fossil, and geological data to support her claims.

L. Sagan. “On the Origin of Mitosing Cells.” *Journal of Theoretical Biology* 14 no. 3 (1967):225–274.

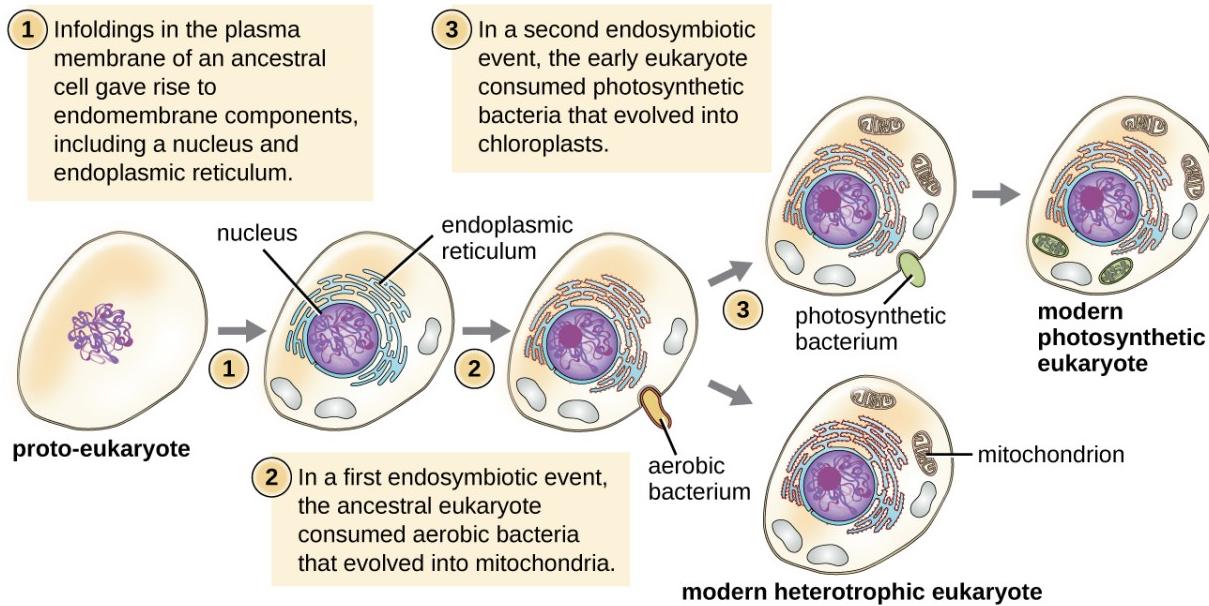
Again, this hypothesis was not initially popular, but mounting genetic evidence due to the advent of DNA sequencing supported the **endosymbiotic theory**, which is now defined as the theory that mitochondria and chloroplasts arose as a result of prokaryotic cells establishing a symbiotic relationship within a eukaryotic host ([\[link\]](#)). With Margulis' initial endosymbiotic theory gaining wide acceptance, she expanded on the theory in her 1981 book *Symbiosis in Cell Evolution*. In it, she explains how endosymbiosis is a major driving factor in the evolution of organisms. More recent genetic sequencing and phylogenetic analysis show that mitochondrial DNA and chloroplast DNA are highly related to their bacterial counterparts, both in DNA sequence and chromosome structure. However, mitochondrial DNA and chloroplast DNA are reduced compared with nuclear DNA because many of the genes have moved from the organelles into the host cell's nucleus. Additionally, mitochondrial and chloroplast ribosomes are structurally similar to bacterial ribosomes, rather than to the eukaryotic ribosomes of their hosts. Last, the binary fission of these organelles strongly resembles the binary fission of bacteria, as compared with mitosis performed by eukaryotic cells. Since Margulis' original proposal, scientists have observed several examples of bacterial endosymbionts in modern-day eukaryotic cells. Examples include the endosymbiotic bacteria found within the guts of certain insects, such as

cockroaches,[[footnote](#)] and photosynthetic bacteria-like organelles found in protists.[[footnote](#)]

A.E. Douglas. "The Microbial Dimension in Insect Nutritional Ecology." *Functional Ecology* 23 (2009):38–47.

J.M. Jaynes, L.P. Vernon. "The Cyanelle of *Cyanophora paradoxa*: Almost a Cyanobacterial Chloroplast." *Trends in Biochemical Sciences* 7 no. 1 (1982):22–24.

The Endosymbiotic Theory



According to the endosymbiotic theory, mitochondria and chloroplasts are each derived from the uptake of bacteria. These bacteria established a symbiotic relationship with their host cell that eventually led to the bacteria evolving into mitochondria and chloroplasts.

Note:

- What does the modern endosymbiotic theory state?

- What evidence supports the endosymbiotic theory?

The Germ Theory of Disease

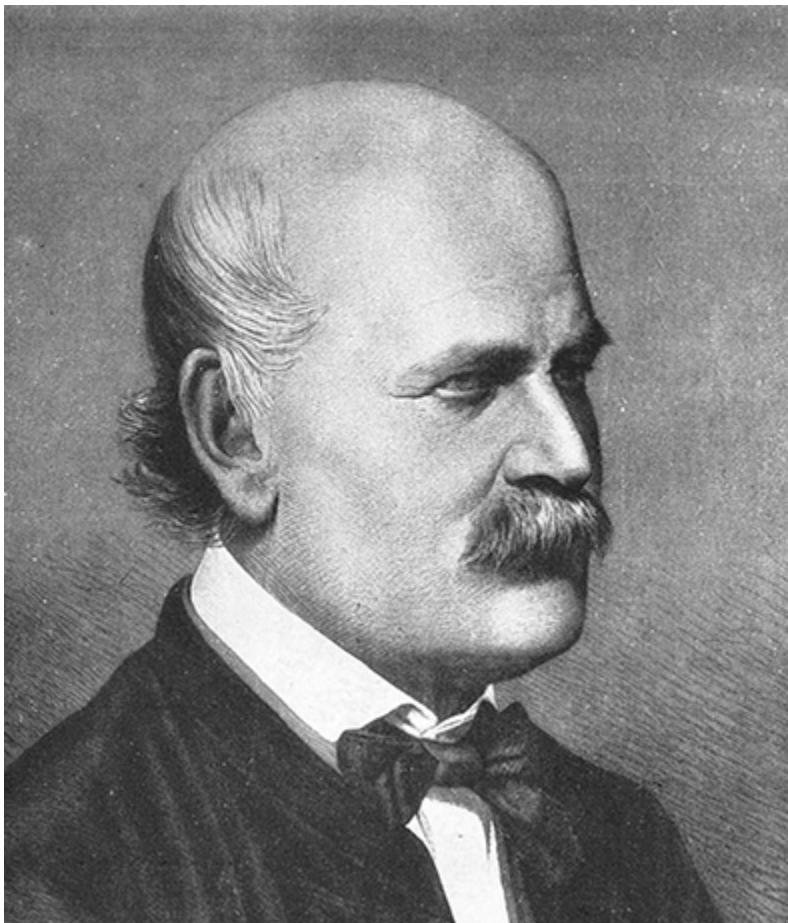
Prior to the discovery of microbes during the 17th century, other theories circulated about the origins of disease. For example, the ancient Greeks proposed the miasma theory, which held that disease originated from particles emanating from decomposing matter, such as that in sewage or cesspits. Such particles infected humans in close proximity to the rotting material. Diseases including the Black Death, which ravaged Europe's population during the Middle Ages, were thought to have originated in this way.

In 1546, Italian physician Girolamo Fracastoro proposed, in his essay *De Contagione et Contagiosis Morbis*, that seed-like spores may be transferred between individuals through direct contact, exposure to contaminated clothing, or through the air. We now recognize Fracastoro as an early proponent of the **germ theory of disease**, which states that diseases may result from microbial infection. However, in the 16th century, Fracastoro's ideas were not widely accepted and would be largely forgotten until the 19th century.

In 1847, Hungarian obstetrician Ignaz Semmelweis ([\[link\]](#)) observed that mothers who gave birth in hospital wards staffed by physicians and medical students were more likely to suffer and die from puerperal fever after childbirth (10%–20% mortality rate) than were mothers in wards staffed by midwives (1% mortality rate). Semmelweis observed medical students performing autopsies and then subsequently carrying out vaginal examinations on living patients without washing their hands in between. He suspected that the students carried disease from the autopsies to the patients they examined. His suspicions were supported by the untimely death of a friend, a physician who contracted a fatal wound infection after a postmortem examination of a woman who had died of a puerperal infection. The dead physician's wound had been caused by a scalpel used during the

examination, and his subsequent illness and death closely paralleled that of the dead patient.

Although Semmelweis did not know the true cause of puerperal fever, he proposed that physicians were somehow transferring the causative agent to their patients. He suggested that the number of puerperal fever cases could be reduced if physicians and medical students simply washed their hands with chlorinated lime water before and after examining every patient. When this practice was implemented, the maternal mortality rate in mothers cared for by physicians dropped to the same 1% mortality rate observed among mothers cared for by midwives. This demonstrated that handwashing was a very effective method for preventing disease transmission. Despite this great success, many discounted Semmelweis's work at the time, and physicians were slow to adopt the simple procedure of handwashing to prevent infections in their patients because it contradicted established norms for that time period.



Ignaz Semmelweis (1818–1865) was a proponent of the importance of handwashing to prevent transfer of disease between patients by physicians.

Around the same time Semmelweis was promoting handwashing, in 1848, British physician John Snow conducted studies to track the source of cholera outbreaks in London. By tracing the outbreaks to two specific water sources, both of which were contaminated by sewage, Snow ultimately demonstrated that cholera bacteria were transmitted via drinking water. Snow's work is influential in that it represents the first known epidemiological study, and it resulted in the first known public health response to an epidemic. The work of both Semmelweis and Snow clearly

refuted the prevailing miasma theory of the day, showing that disease is not only transmitted through the air but also through contaminated items.

Although the work of Semmelweis and Snow successfully showed the role of sanitation in preventing infectious disease, the cause of disease was not fully understood. The subsequent work of Louis Pasteur, Robert Koch, and Joseph Lister would further substantiate the germ theory of disease.

While studying the causes of beer and wine spoilage in 1856, Pasteur discovered properties of fermentation by microorganisms. He had demonstrated with his swan-neck flask experiments ([\[link\]](#)) that airborne microbes, not spontaneous generation, were the cause of food spoilage, and he suggested that if microbes were responsible for food spoilage and fermentation, they could also be responsible for causing infection. This was the foundation for the germ theory of disease.

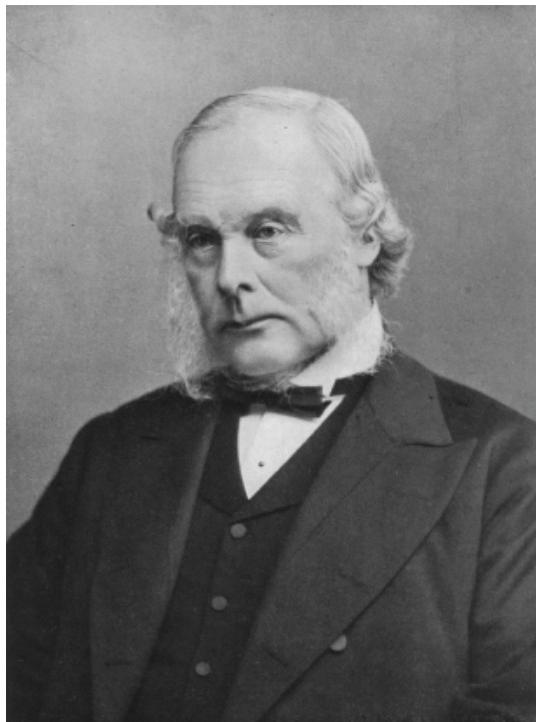
Meanwhile, British surgeon Joseph Lister ([\[link\]](#)) was trying to determine the causes of postsurgical infections. Many physicians did not give credence to the idea that microbes on their hands, on their clothes, or in the air could infect patients' surgical wounds, despite the fact that 50% of surgical patients, on average, were dying of postsurgical infections.

[\[footnote\]](#) Lister, however, was familiar with the work of Semmelweis and Pasteur; therefore, he insisted on handwashing and extreme cleanliness during surgery. In 1867, to further decrease the incidence of postsurgical wound infections, Lister began using carbolic acid (phenol) spray disinfectant/antiseptic during surgery. His extremely successful efforts to reduce postsurgical infection caused his techniques to become a standard medical practice.

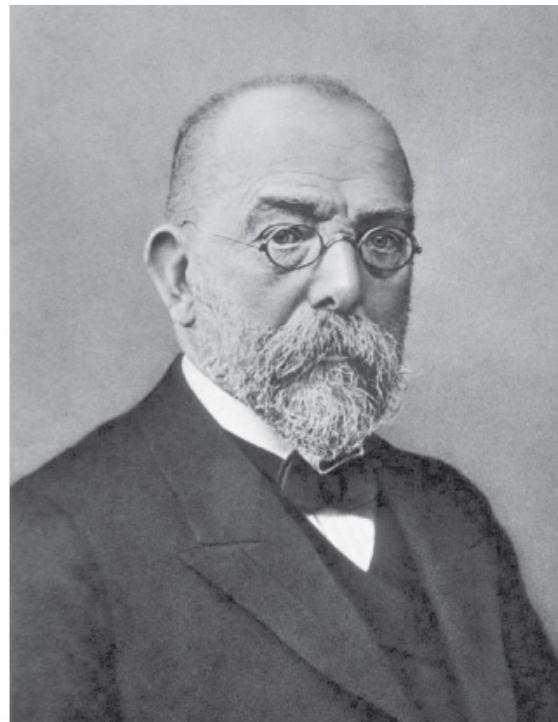
Alexander, J. Wesley. "The Contributions of Infection Control to a Century of Progress" *Annals of Surgery* 201:423-428, 1985.

A few years later, Robert Koch ([\[link\]](#)) proposed a series of postulates (Koch's postulates) based on the idea that the cause of a specific disease could be attributed to a specific microbe. Using these postulates, Koch and his colleagues were able to definitively identify the causative pathogens of specific diseases, including anthrax, tuberculosis, and cholera. Koch's "one microbe, one disease" concept was the culmination of the 19th century's paradigm shift away from miasma theory and toward the germ theory of

disease. Koch's postulates are discussed more thoroughly in [How Pathogens Cause Disease](#).



(a)



(b)

(a) Joseph Lister developed procedures for the proper care of surgical wounds and the sterilization of surgical equipment. (b) Robert Koch established a protocol to determine the cause of infectious disease. Both scientists contributed significantly to the acceptance of the germ theory of disease.

Note:

- Compare and contrast the miasma theory of disease with the germ theory of disease.

- How did Joseph Lister's work contribute to the debate between the miasma theory and germ theory and how did this increase the success of medical procedures?

Note:

Part 2

After suffering a fever, congestion, cough, and increasing aches and pains for several days, Barbara suspects that she has a case of the flu. She decides to visit the health center at her university. The PA tells Barbara that her symptoms could be due to a range of diseases, such as influenza, bronchitis, pneumonia, or tuberculosis.

During her physical examination, the PA notes that Barbara's heart rate is slightly elevated. Using a pulse oximeter, a small device that clips on her finger, he finds that Barbara has hypoxemia—a lower-than-normal level of oxygen in the blood. Using a stethoscope, the PA listens for abnormal sounds made by Barbara's heart, lungs, and digestive system. As Barbara breathes, the PA hears a crackling sound and notes a slight shortness of breath. He collects a sputum sample, noting the greenish color of the mucus, and orders a chest radiograph, which shows a “shadow” in the left lung. All of these signs are suggestive of pneumonia, a condition in which the lungs fill with mucus ([\[link\]](#)).



lung infiltrated, suggestive of pneumonia

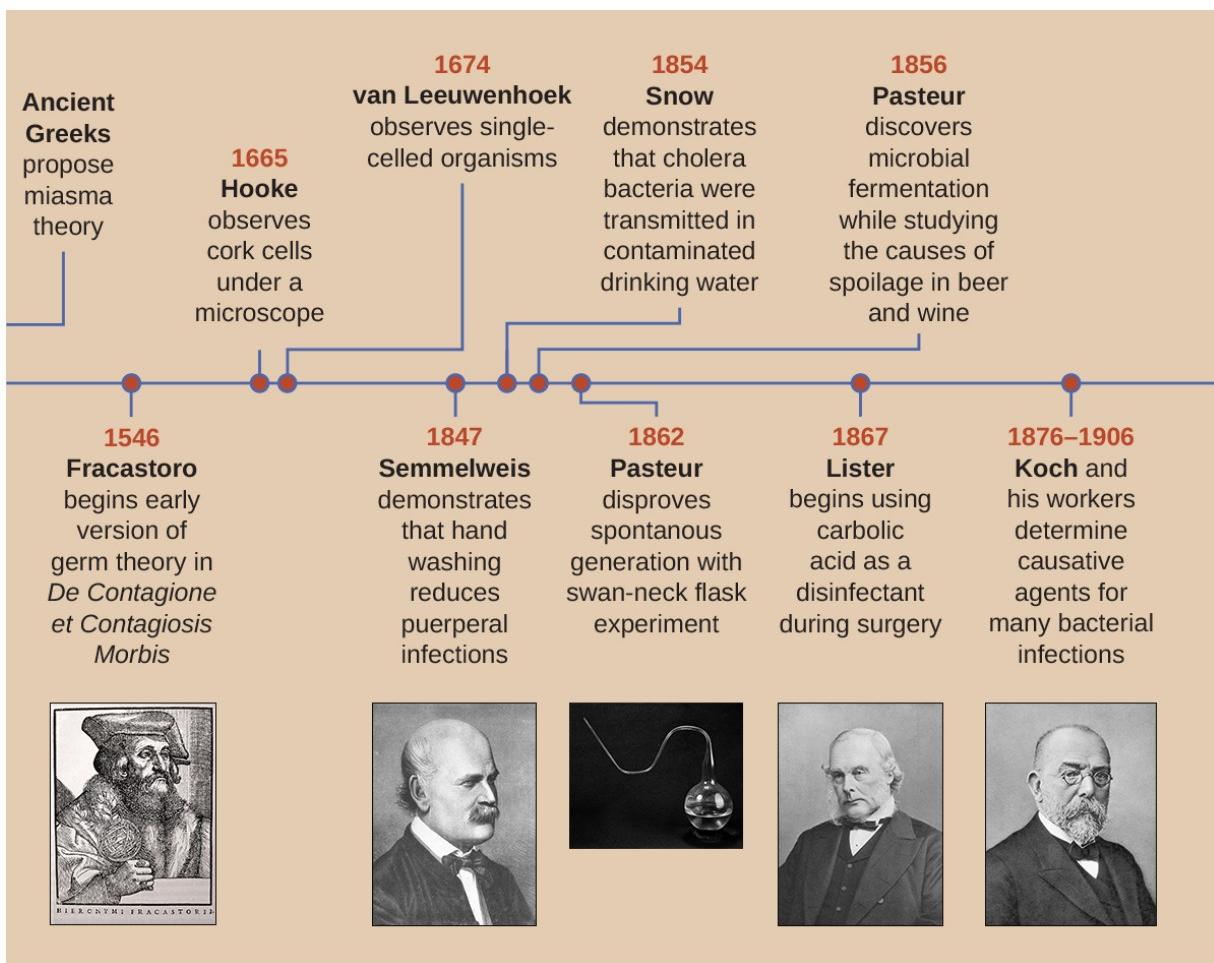


normal lungs

This is a chest radiograph typical of pneumonia. Because X-ray images are negative images, a “shadow” is seen as a white area within the lung that should otherwise be black. In this case, the left lung shows a shadow as a result of pockets in the lung that have become filled with fluid. (credit left: modification of work by “Christaras A”/Wikimedia Commons)

- What kinds of infectious agents are known to cause pneumonia?

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.



(credit “swan-neck flask”: modification of work by Wellcome Images)

Key Concepts and Summary

- Although cells were first observed in the 1660s by Robert Hooke, **cell theory** was not well accepted for another 200 years. The work of scientists such as Schleiden, Schwann, Remak, and Virchow contributed to its acceptance.
- **Endosymbiotic theory** states that mitochondria and chloroplasts, organelles found in many types of organisms, have their origins in bacteria. Significant structural and genetic information support this theory.
- The **miasma theory of disease** was widely accepted until the 19th century, when it was replaced by the **germ theory of disease** thanks to the work of Semmelweis, Snow, Pasteur, Lister, and Koch, and others.

Critical Thinking

Exercise:

Problem:

Why are mitochondria and chloroplasts unable to multiply outside of a host cell?

Exercise:

Problem:

Why was the work of Snow so important in supporting the germ theory?

Unique Characteristics of Prokaryotic Cells

LEARNING OBJECTIVES

- Explain the distinguishing characteristics of prokaryotic cells
- Describe common cell morphologies and cellular arrangements typical of prokaryotic cells and explain how cells maintain their morphology
- Describe internal and external structures of prokaryotic cells in terms of their physical structure, chemical structure, and function
- Compare the distinguishing characteristics of bacterial and archaeal cells

Cell theory states that the cell is the fundamental unit of life. However, cells vary significantly in size, shape, structure, and function. At the simplest level of construction, all cells possess a few fundamental components. These include **cytoplasm** (a gel-like substance composed of water and dissolved chemicals needed for growth), which is contained within a plasma membrane (also called a cell membrane or cytoplasmic membrane); one or more chromosomes, which contain the genetic blueprints of the cell; and **ribosomes**, organelles used for the production of proteins.

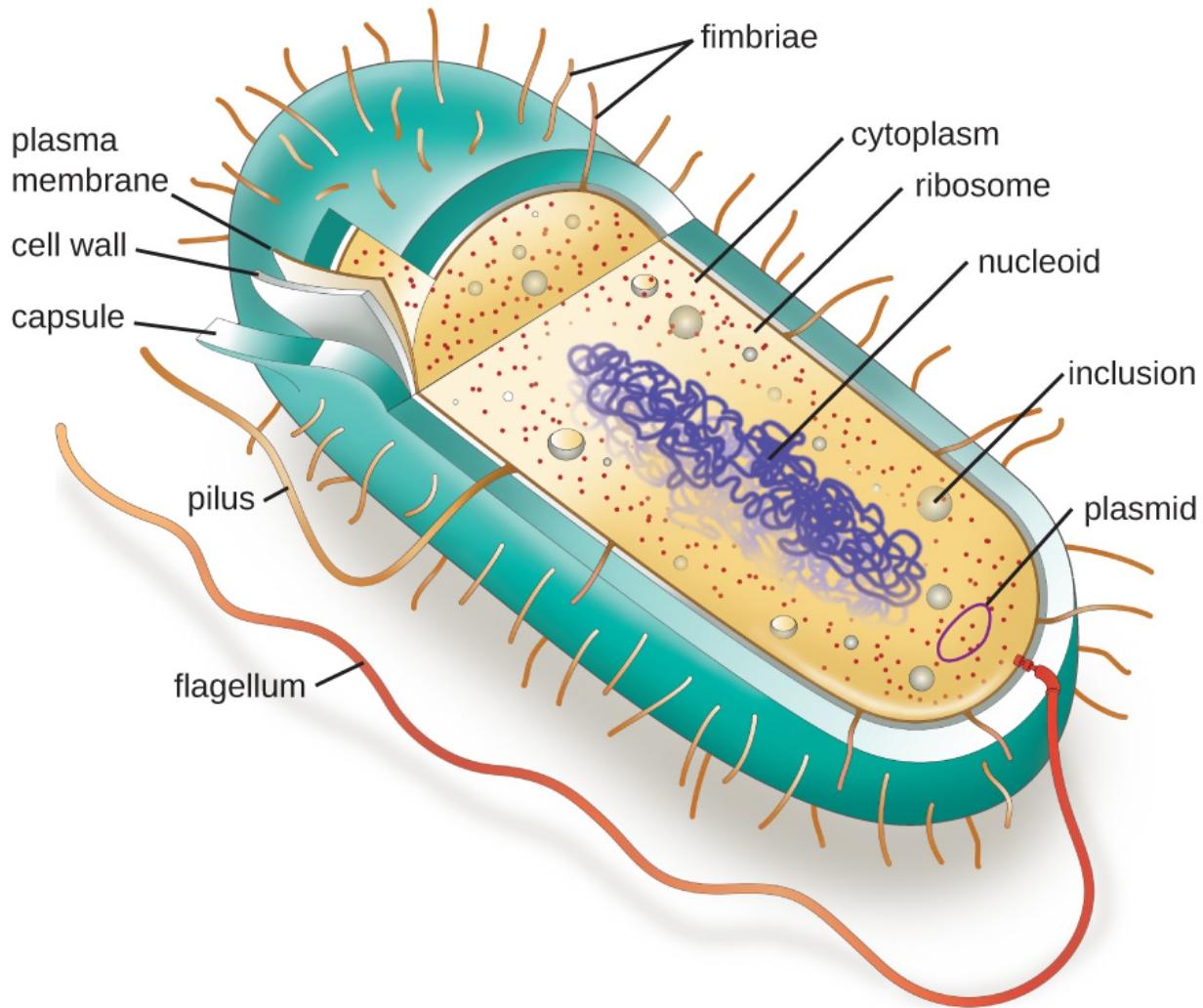
Beyond these basic components, cells can vary greatly between organisms, and even within the same multicellular organism. The two largest categories of cells—**prokaryotic cells** and **eukaryotic cells**—are defined by major differences in several cell structures. Prokaryotic cells lack a nucleus surrounded by a complex nuclear membrane and generally have a single, circular chromosome located in a nucleoid. Eukaryotic cells have a nucleus

surrounded by a complex nuclear membrane that contains multiple, rod-shaped chromosomes.[\[footnote\]](#)

Y.-H.M. Chan, W.F. Marshall. “Scaling Properties of Cell and Organelle Size.” *Organogenesis* 6 no. 2 (2010):88–96.

All plant cells and animal cells are eukaryotic. Some microorganisms are composed of prokaryotic cells, whereas others are composed of eukaryotic cells. Prokaryotic microorganisms are classified within the domains Archaea and Bacteria, whereas eukaryotic organisms are classified within the domain Eukarya.

The structures inside a cell are analogous to the organs inside a human body, with unique structures suited to specific functions. Some of the structures found in prokaryotic cells are similar to those found in some eukaryotic cells; others are unique to prokaryotes. Although there are some exceptions, eukaryotic cells tend to be larger than prokaryotic cells. The comparatively larger size of eukaryotic cells dictates the need to compartmentalize various chemical processes within different areas of the cell, using complex membrane-bound organelles. In contrast, prokaryotic cells generally lack membrane-bound organelles; however, they often contain inclusions that compartmentalize their cytoplasm. [\[link\]](#) illustrates structures typically associated with prokaryotic cells. These structures are described in more detail in the next section.

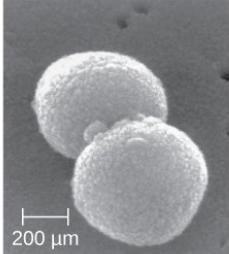
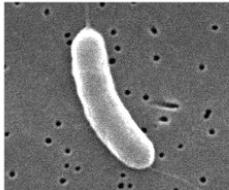
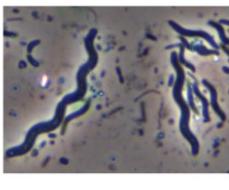


A typical prokaryotic cell contains a cell membrane, chromosomal DNA that is concentrated in a nucleoid, ribosomes, and a cell wall. Some prokaryotic cells may also possess flagella, pili, fimbriae, and capsules.

Common Cell Morphologies and Arrangements

Individual cells of a particular prokaryotic organism are typically similar in shape, or **cell morphology**. Although thousands of prokaryotic organisms have been identified, only a handful of cell morphologies are commonly seen microscopically. [\[link\]](#) names and illustrates cell morphologies

commonly found in prokaryotic cells. In addition to cellular shape, prokaryotic cells of the same species may group together in certain distinctive arrangements depending on the plane of cell division. Some common arrangements are shown in [[link](#)].

Common Prokaryotic Cell Shapes			
Name	Description	Illustration	Image
Coccus (pl. cocci)	Round		
Bacillus (pl. bacilli)	Rod		
Vibrio (pl. vibrios)	Curved rod		
Coccobacillus (pl. coccobacilli)	Short rod		
Spirillum (pl. spirilla)	Spiral		
Spirochete (pl. spirochetes)	Long, loose, helical spiral		

(credit “Coccus” micrograph: modification of work by Janice Haney

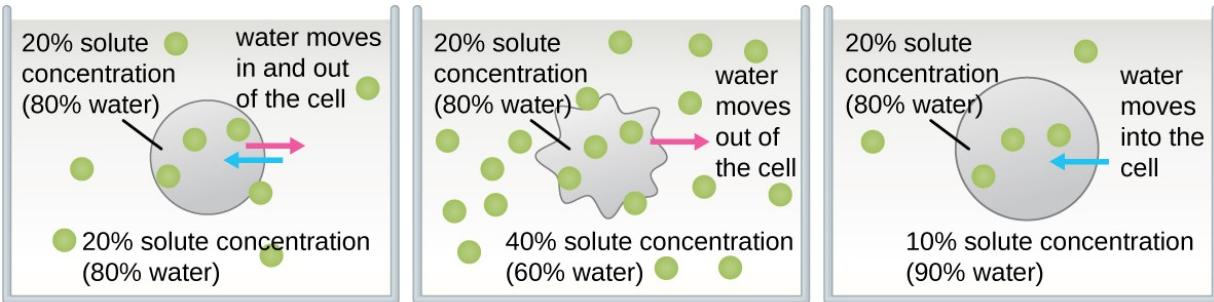
Carr, Centers for Disease Control and Prevention; credit “Coccobacillus” micrograph: modification of work by Janice Carr, Centers for Disease Control and Prevention; credit “Spirochete” micrograph: modification of work by Centers for Disease Control and Prevention)

Common Prokaryotic Cell Arrangements		
Name	Description	Illustration
Coccus (pl. cocci)	Single coccus	
Diplococcus (pl. diplococci)	Pair of two cocci	
Tetrad (pl. tetrads)	Grouping of four cells arranged in a square	
Streptococcus (pl. streptococci)	Chain of cocci	
Staphylococcus (pl. staphylococci)	Cluster of cocci	
Bacillus (pl. bacilli)	Single rod	
Streptobacillus (pl. streptobacilli)	Chain of rods	

In most prokaryotic cells, morphology is maintained by the **cell wall** in combination with cytoskeletal elements. The cell wall is a structure found in most prokaryotes and some eukaryotes; it envelopes the cell membrane, protecting the cell from changes in **osmotic pressure** ([\[link\]](#)). Osmotic pressure occurs because of differences in the concentration of solutes on opposing sides of a semipermeable membrane. Water is able to pass through a semipermeable membrane, but solutes (dissolved molecules like salts, sugars, and other compounds) cannot. When the concentration of solutes is greater on one side of the membrane, water diffuses across the membrane from the side with the lower concentration (more water) to the side with the higher concentration (less water) until the concentrations on both sides become equal. This diffusion of water is called **osmosis**, and it can cause extreme osmotic pressure on a cell when its external environment changes.

The external environment of a cell can be described as an isotonic, hypertonic, or hypotonic medium. In an **isotonic medium**, the solute concentrations inside and outside the cell are approximately equal, so there is no net movement of water across the cell membrane. In a **hypertonic medium**, the solute concentration outside the cell exceeds that inside the cell, so water diffuses out of the cell and into the external medium. In a **hypotonic medium**, the solute concentration inside the cell exceeds that outside of the cell, so water will move by osmosis into the cell. This causes the cell to swell and potentially lyse, or burst.

The degree to which a particular cell is able to withstand changes in osmotic pressure is called tonicity. Cells that have a cell wall are better able to withstand subtle changes in osmotic pressure and maintain their shape. In hypertonic environments, cells that lack a cell wall can become dehydrated, causing **crenation**, or shriveling of the cell; the plasma membrane contracts and appears scalloped or notched ([\[link\]](#)). By contrast, cells that possess a cell wall undergo **plasmolysis** rather than crenation. In plasmolysis, the plasma membrane contracts and detaches from the cell wall, and there is a decrease in interior volume, but the cell wall remains intact, thus allowing the cell to maintain some shape and integrity for a period of time ([\[link\]](#)). Likewise, cells that lack a cell wall are more prone to lysis in hypotonic environments. The presence of a cell wall allows the cell to maintain its shape and integrity for a longer time before lysing ([\[link\]](#)).



a Isotonic solution

A solution that has the *same* solute concentration as another solution. There is no net movement of water particles, and the overall concentration on both sides of the cell membrane remains constant.

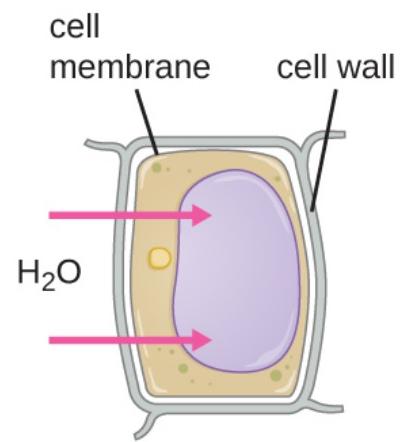
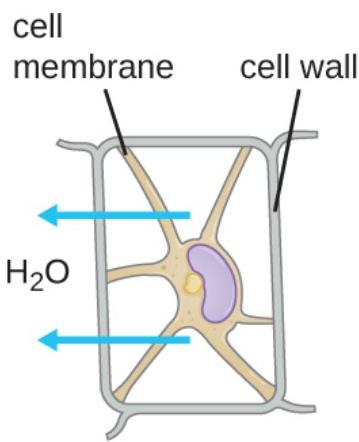
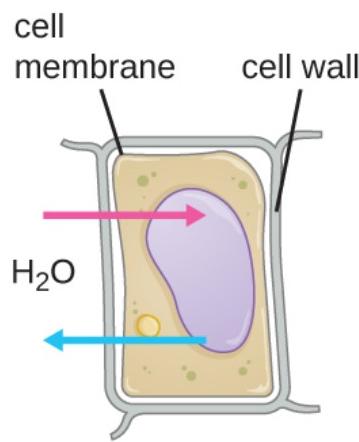
b Hypertonic solution

A solution that has a *higher* solute concentration than another solution. Water particles will move out of the cell, causing crenation.

c Hypotonic solution

A solution that has a *lower* solute concentration than another solution. Water particles will move into the cell, causing the cell to expand and eventually lyse.

In cells that lack a cell wall, changes in osmotic pressure can lead to crenation in hypertonic environments or cell lysis in hypotonic environments.



a Isotonic solution

No net movement of water particles. Cell membrane is attached to cell wall.

b Hypertonic solution

Water particles move out of the cell. Cell membrane shrinks and detaches from cell wall (plasmolysis).

c Hypotonic solution

Water particles move into the cell. Cell wall counteracts osmotic pressure to prevent swelling and lysis.

In prokaryotic cells, the cell wall provides some protection against changes in osmotic pressure, allowing it to maintain its shape longer. The cell membrane is typically attached to the cell wall in an isotonic medium (left). In a hypertonic medium, the cell membrane detaches from the cell wall and contracts (plasmolysis) as water leaves the cell.

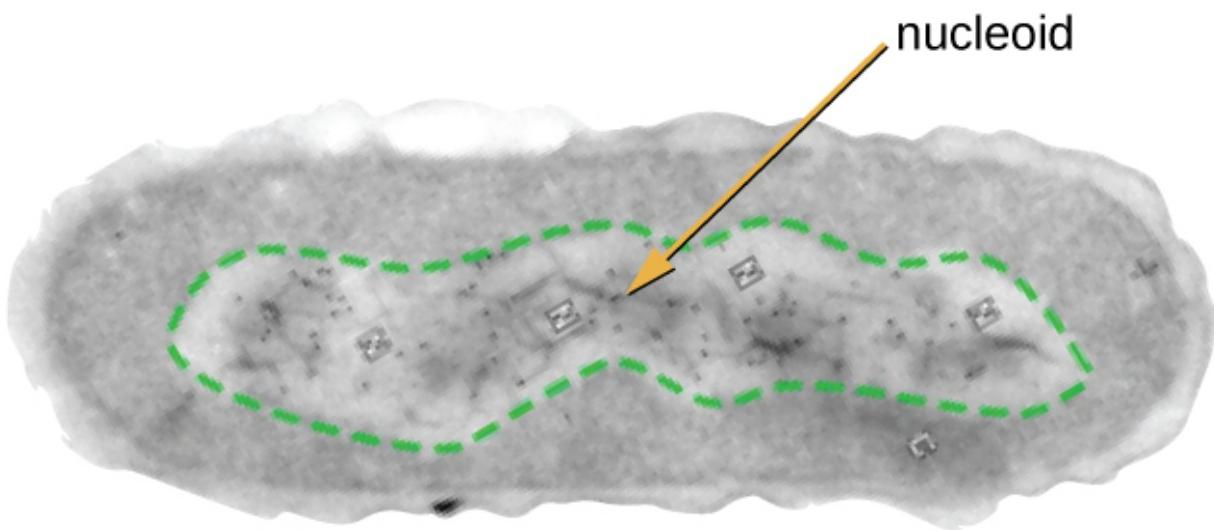
In a hypotonic medium (right), the cell wall prevents the cell membrane from expanding to the point of bursting, although lysis will eventually occur if too much water is absorbed.

Note:

- Explain the difference between cell morphology and arrangement.
- What advantages do cell walls provide prokaryotic cells?

The Nucleoid

All cellular life has a DNA genome organized into one or more chromosomes. Prokaryotic chromosomes are typically circular, haploid (unpaired), and not bound by a complex nuclear membrane. Prokaryotic DNA and DNA-associated proteins are concentrated within the **nucleoid** region of the cell ([\[link\]](#)). In general, prokaryotic DNA interacts with **nucleoid-associated proteins (NAPs)** that assist in the organization and packaging of the chromosome. In bacteria, NAPs function similar to histones, which are the DNA-organizing proteins found in eukaryotic cells. In archaea, the nucleoid is organized by either NAPs or histone-like DNA organizing proteins.



The nucleoid region (the area enclosed by the green dashed line) is a condensed area of DNA found within prokaryotic cells. Because of the density of the area, it does not readily stain and appears lighter in color when viewed with a transmission electron microscope.

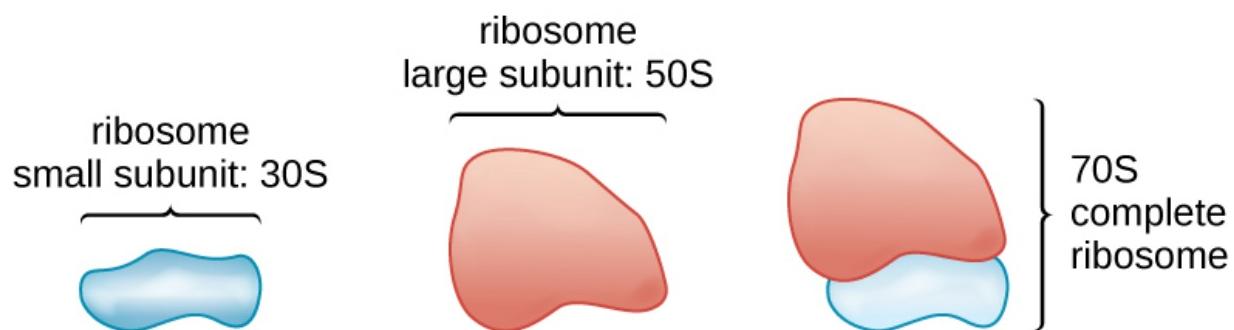
Plasmids

Prokaryotic cells may also contain extrachromosomal DNA, or DNA that is not part of the chromosome. This extrachromosomal DNA is found in **plasmids**, which are small, circular, double-stranded DNA molecules. Cells that have plasmids often have hundreds of them within a single cell. Plasmids are more commonly found in bacteria; however, plasmids have been found in archaea and eukaryotic organisms. Plasmids often carry genes that confer advantageous traits such as antibiotic resistance; thus, they are important to the survival of the organism. We will discuss plasmids in more detail in [Mechanisms of Microbial Genetics](#).

Ribosomes

All cellular life synthesizes proteins, and organisms in all three domains of life possess ribosomes, structures responsible for protein synthesis. However, ribosomes in each of the three domains are structurally different.

Ribosomes, themselves, are constructed from proteins, along with ribosomal RNA (rRNA). Prokaryotic ribosomes are found in the cytoplasm. They are called **70S ribosomes** because they have a size of 70S ([\[link\]](#)), whereas eukaryotic cytoplasmic ribosomes have a size of 80S. (The S stands for Svedberg unit, a measure of sedimentation in an ultracentrifuge, which is based on size, shape, and surface qualities of the structure being analyzed). Although they are the same size, bacterial and archaeal ribosomes have different proteins and rRNA molecules, and the archaeal versions are more similar to their eukaryotic counterparts than to those found in bacteria.



Prokaryotic ribosomes (70S) are composed of two subunits: the 30S

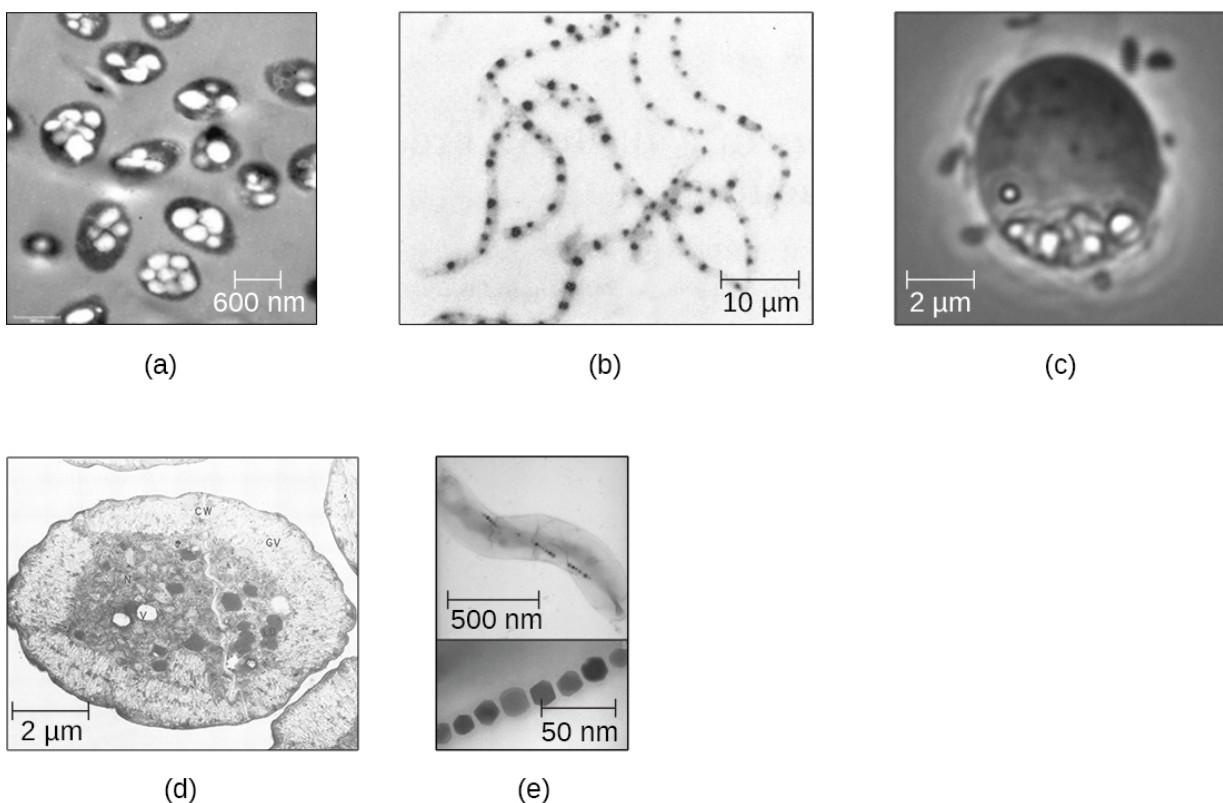
(small subunit) and the 50S (large subunit), each of which are composed of protein and rRNA components.

Inclusions

As single-celled organisms living in unstable environments, some prokaryotic cells have the ability to store excess nutrients within cytoplasmic structures called **inclusions**. Storing nutrients in a polymerized form is advantageous because it reduces the buildup of osmotic pressure that occurs as a cell accumulates solutes. Various types of inclusions store glycogen and starches, which contain carbon that cells can access for energy. **Volutin** granules, also called **metachromatic granules** because of their staining characteristics, are inclusions that store polymerized inorganic phosphate that can be used in metabolism and assist in the formation of biofilms. Microbes known to contain volutin granules include the archaea *Methanosarcina*, the bacterium *Corynebacterium diphtheriae*, and the unicellular eukaryotic alga *Chlamydomonas*. Sulfur granules, another type of inclusion, are found in sulfur bacteria of the genus *Thiobacillus*; these granules store elemental sulfur, which the bacteria use for metabolism.

Occasionally, certain types of inclusions are surrounded by a phospholipid monolayer embedded with protein. **Polyhydroxybutyrate (PHB)**, which can be produced by species of *Bacillus* and *Pseudomonas*, is an example of an inclusion that displays this type of monolayer structure. Industrially, PHB has also been used as a source of biodegradable polymers for bioplastics. Several different types of inclusions are shown in [\[link\]](#).

prokaryote inclusion bodies



Prokaryotic cells may have various types of inclusions. (a) A transmission electron micrograph of polyhydroxybutyrate lipid droplets. (b) A light micrograph of volutin granules. (c) A phase-contrast micrograph of sulfur granules. (d) A transmission electron micrograph of magnetosomes. (e) A transmission electron micrograph of gas vacuoles. (credit b, c, d: modification of work by American Society for Microbiology)

Some prokaryotic cells have other types of inclusions that serve purposes other than nutrient storage. For example, some prokaryotic cells produce gas vacuoles, accumulations of small, protein-lined vesicles of gas. These gas vacuoles allow the prokaryotic cells that synthesize them to alter their buoyancy so that they can adjust their location in the water column. Magnetotactic bacteria, such as *Magnetospirillum magnetotacticum*, contain **magnetosomes**, which are inclusions of magnetic iron oxide or iron

sulfide surrounded by a lipid layer. These allow cells to align along a magnetic field, aiding their movement ([\[link\]](#)). Cyanobacteria such as *Anabaena cylindrica* and bacteria such as *Halothiobacillus neapolitanus* produce **carboxysome** inclusions. Carboxysomes are composed of outer shells of thousands of protein subunits. Their interior is filled with ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and carbonic anhydrase. Both of these compounds are used for carbon metabolism. Some prokaryotic cells also possess carboxysomes that sequester functionally related enzymes in one location. These structures are considered proto-organelles because they compartmentalize important compounds or chemical reactions, much like many eukaryotic organelles.

Endospores

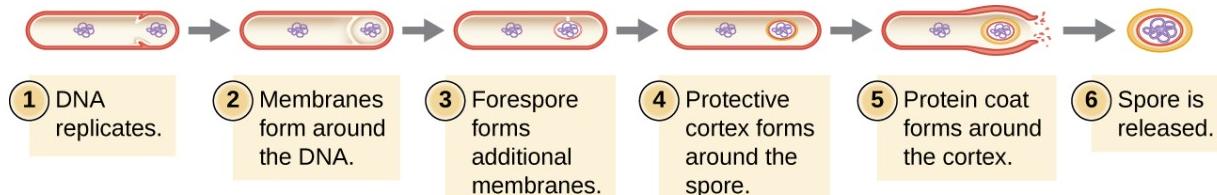
Bacterial cells are generally observed as **vegetative cells**, but some genera of bacteria have the ability to form **endospores**, structures that essentially protect the bacterial genome in a dormant state when environmental conditions are unfavorable. Endospores (not to be confused with the reproductive spores formed by fungi) allow some bacterial cells to survive long periods without food or water, as well as exposure to chemicals, extreme temperatures, and even radiation. [\[link\]](#) compares the characteristics of vegetative cells and endospores.

Characteristics of Vegetative Cells versus Endospores	
Vegetative Cells	Endospores
Sensitive to extreme temperatures and radiation	Resistant to extreme temperatures and radiation

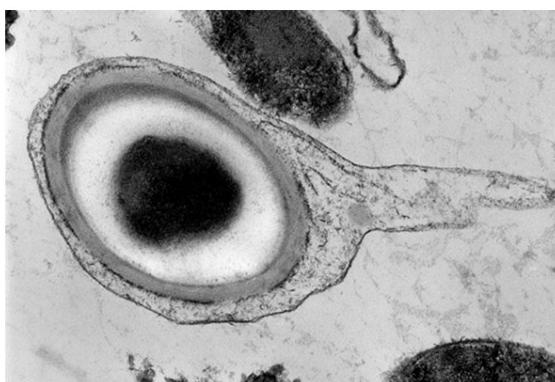
Characteristics of Vegetative Cells versus Endospores

Vegetative Cells	Endospores
Gram-positive	Do not absorb Gram stain, only special endospore stains (see Staining Microscopic Specimens)
Normal water content and enzymatic activity	Dehydrated; no metabolic activity
Capable of active growth and metabolism	Dormant; no growth or metabolic activity

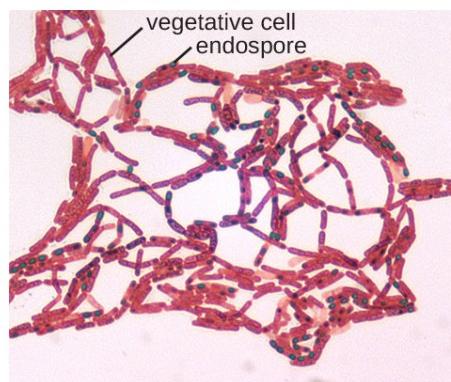
The process by which vegetative cells transform into endospores is called **sporulation**, and it generally begins when nutrients become depleted or environmental conditions become otherwise unfavorable ([\[link\]](#)). The process begins with the formation of a septum in the vegetative bacterial cell. The septum divides the cell asymmetrically, separating a DNA forespore from the mother cell. The forespore, which will form the core of the endospore, is essentially a copy of the cell's chromosomes, and is separated from the mother cell by a second membrane. A cortex gradually forms around the forespore by laying down layers of calcium and dipicolinic acid between membranes. A protein spore coat then forms around the cortex while the DNA of the mother cell disintegrates. Further maturation of the endospore occurs with the formation of an outermost exosporium. The endospore is released upon disintegration of the mother cell, completing sporulation.



(a)



(b)



(c)

(a) Sporulation begins following asymmetric cell division. The forespore becomes surrounded by a double layer of membrane, a cortex, and a protein spore coat, before being released as a mature endospore upon disintegration of the mother cell. (b) An electron micrograph of a *Carboxydothermus hydrogenoformans* endospore. (c) These *Bacillus* spp. cells are undergoing sporulation. The endospores have been visualized using Malachite Green spore stain. (credit b: modification of work by Jonathan Eisen)

Endospores of certain species have been shown to persist in a dormant state for extended periods of time, up to thousands of years.[\[footnote\]](#) However, when living conditions improve, endospores undergo **germination**, reentering a vegetative state. After germination, the cell becomes metabolically active again and is able to carry out all of its normal functions, including growth and cell division.

F. Rothfuss, M Bender, R Conrad. "Survival and Activity of Bacteria in a Deep, Aged Lake Sediment (Lake Constance)." *Microbial Ecology* 33 no. 1 (1997):69–77.

Not all bacteria have the ability to form endospores; however, there are a number of clinically significant endospore-forming gram-positive bacteria of the genera *Bacillus* and *Clostridium*. These include *B. anthracis*, the causative agent of anthrax, which produces endospores capable of surviving for many decades [footnote]; *C. tetani* (causes tetanus); *C. difficile* (causes pseudomembranous colitis); *C. perfringens* (causes gas gangrene); and *C. botulinum* (causes botulism). Pathogens such as these are particularly difficult to combat because their endospores are so hard to kill. Special sterilization methods for endospore-forming bacteria are discussed in [Control of Microbial Growth](#).

R. Sinclair et al. "Persistence of Category A Select Agents in the Environment." *Applied and Environmental Microbiology* 74 no. 3 (2008):555–563.

Note:

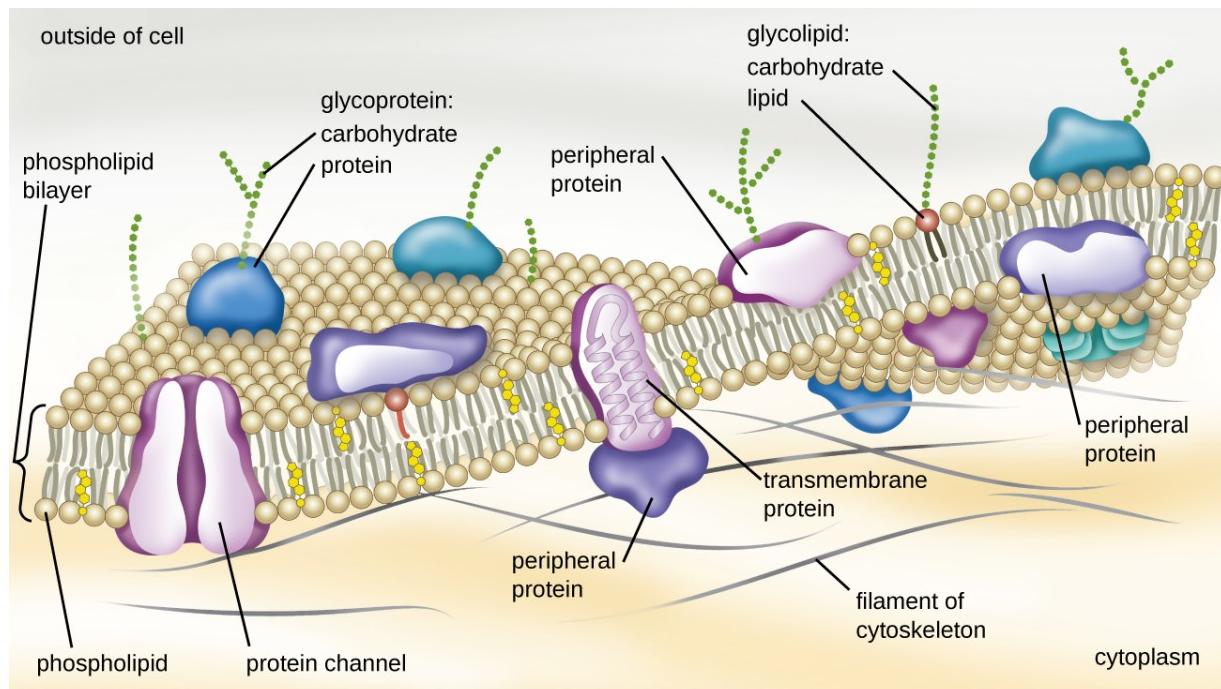
- What is an inclusion?
- What is the function of an endospore?

Plasma Membrane

Structures that enclose the cytoplasm and internal structures of the cell are known collectively as the **cell envelope**. In prokaryotic cells, the structures of the cell envelope vary depending on the type of cell and organism. Most (but not all) prokaryotic cells have a cell wall, but the makeup of this cell wall varies. All cells (prokaryotic and eukaryotic) have a **plasma membrane** (also called **cytoplasmic membrane** or **cell membrane**) that exhibits selective permeability, allowing some molecules to enter or leave the cell while restricting the passage of others.

The structure of the plasma membrane is often described in terms of the **fluid mosaic model**, which refers to the ability of membrane components to move fluidly within the plane of the membrane, as well as the mosaic-like

composition of the components, which include a diverse array of lipid and protein components ([\[link\]](#)). The plasma membrane structure of most bacterial and eukaryotic cell types is a bilayer composed mainly of phospholipids formed with ester linkages and proteins. These phospholipids and proteins have the ability to move laterally within the plane of the membranes as well as between the two phospholipid layers.



The bacterial plasma membrane is a phospholipid bilayer with a variety of embedded proteins that perform various functions for the cell. Note the presence of glycoproteins and glycolipids, whose carbohydrate components extend out from the surface of the cell. The abundance and arrangement of these proteins and lipids can vary greatly between species.

Archaeal membranes are fundamentally different from bacterial and eukaryotic membranes in a few significant ways. First, archaeal membrane phospholipids are formed with ether linkages, in contrast to the ester

linkages found in bacterial or eukaryotic cell membranes. Second, archaeal phospholipids have branched chains, whereas those of bacterial and eukaryotic cells are straight chained. Finally, although some archaeal membranes can be formed of bilayers like those found in bacteria and eukaryotes, other archaeal plasma membranes are lipid monolayers.

Proteins on the cell's surface are important for a variety of functions, including cell-to-cell communication, and sensing environmental conditions and pathogenic virulence factors. Membrane proteins and phospholipids may have carbohydrates (sugars) associated with them and are called glycoproteins or glycolipids, respectively. These glycoprotein and glycolipid complexes extend out from the surface of the cell, allowing the cell to interact with the external environment ([\[link\]](#)). Glycoproteins and glycolipids in the plasma membrane can vary considerably in chemical composition among archaea, bacteria, and eukaryotes, allowing scientists to use them to characterize unique species.

Plasma membranes from different cells types also contain unique phospholipids, which contain fatty acids. As described in [Using Biochemistry to Identify Microorganisms](#), phospholipid-derived fatty acid analysis (PLFA) profiles can be used to identify unique types of cells based on differences in fatty acids. Archaea, bacteria, and eukaryotes each have a unique PFLA profile.

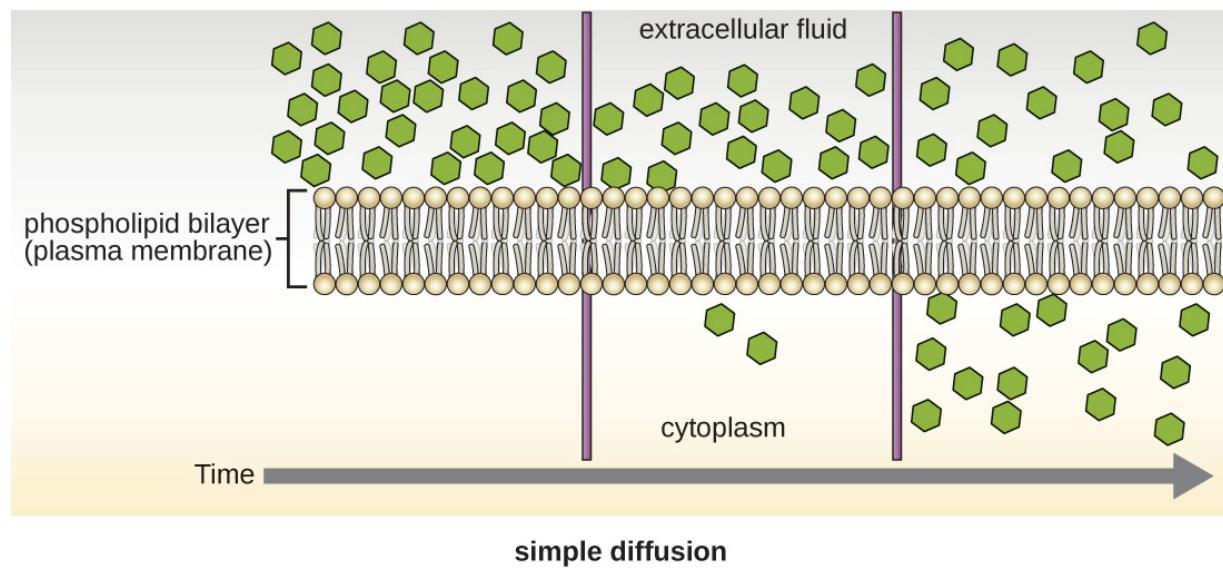
Membrane Transport Mechanisms

One of the most important functions of the plasma membrane is to control the transport of molecules into and out of the cell. Internal conditions must be maintained within a certain range despite any changes in the external environment. The transport of substances across the plasma membrane allows cells to do so.

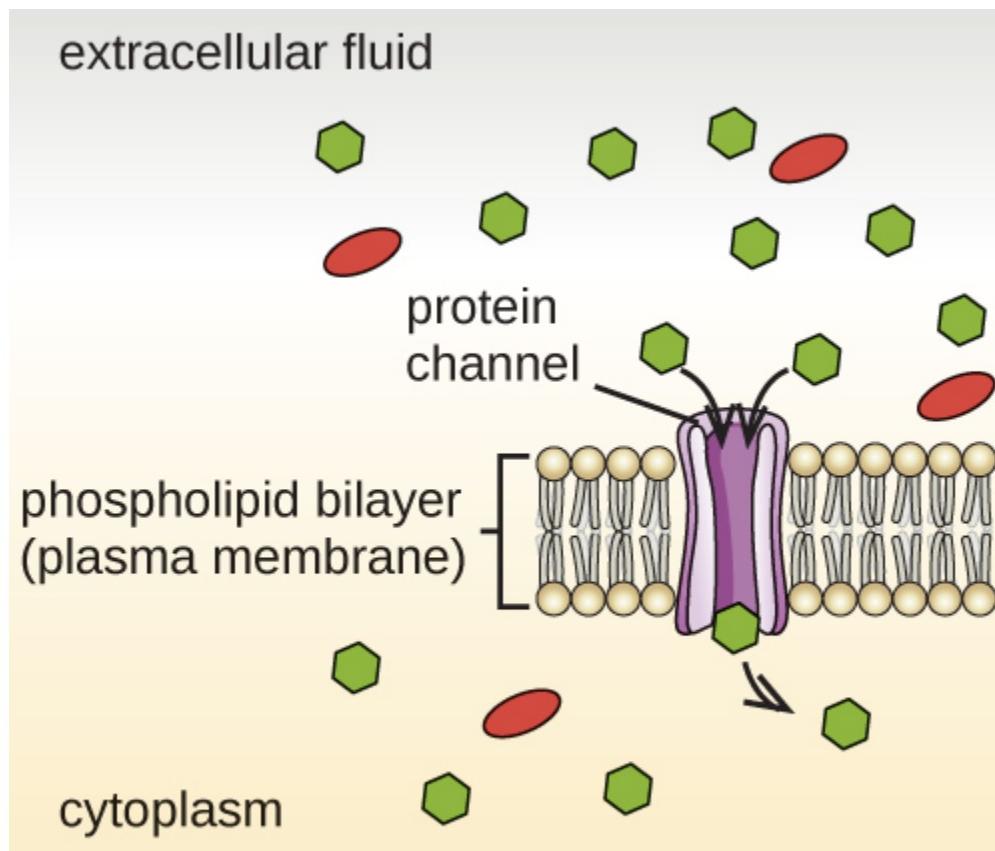
Cells use various modes of transport across the plasma membrane. For example, molecules moving from a higher concentration to a lower concentration with the concentration gradient are transported by simple diffusion, also known as passive transport ([\[link\]](#)). Some small molecules,

like carbon dioxide, may cross the membrane bilayer directly by simple diffusion. However, charged molecules, as well as large molecules, need the help of carriers or channels in the membrane. These structures ferry molecules across the membrane, a process known as facilitated diffusion ([\[link\]](#)).

Active transport occurs when cells move molecules across their membrane *against* concentration gradients ([\[link\]](#)). A major difference between passive and active transport is that active transport requires adenosine triphosphate (ATP) or other forms of energy to move molecules “uphill.” Therefore, active transport structures are often called “pumps.”

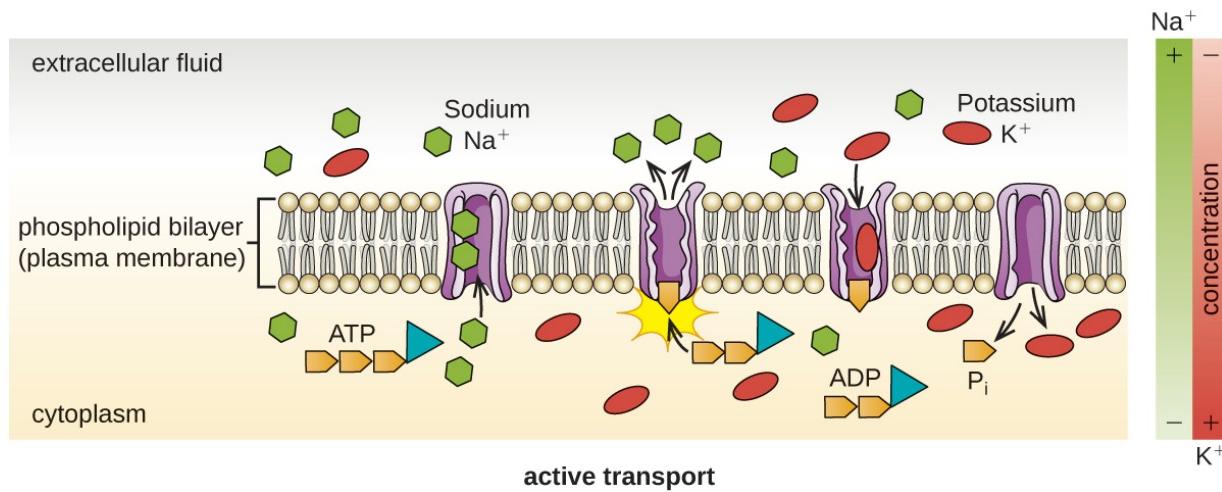


Simple diffusion down a concentration gradient directly across the phospholipid bilayer. (credit: modification of work by Mariana Ruiz Villareal)



facilitated diffusion

Facilitated diffusion down a concentration gradient through a membrane protein. (credit: modification of work by Mariana Ruiz Villareal)



Active transport against a concentration gradient via a membrane pump that requires energy. (credit: modification of work by Mariana Ruiz Villareal)

Group translocation also transports substances into bacterial cells. In this case, as a molecule moves into a cell against its concentration gradient, it is chemically modified so that it does not require transport against an unfavorable concentration gradient. A common example of this is the bacterial phosphotransferase system, a series of carriers that phosphorylates (i.e., adds phosphate ions to) glucose or other sugars upon entry into cells. Since the phosphorylation of sugars is required during the early stages of sugar metabolism, the phosphotransferase system is considered to be an energy neutral system.

Photosynthetic Membrane Structures

Some prokaryotic cells, namely cyanobacteria and photosynthetic bacteria, have membrane structures that enable them to perform photosynthesis. These structures consist of an infolding of the plasma membrane that encloses photosynthetic pigments such as green **chlorophylls** and bacteriochlorophylls. In cyanobacteria, these membrane structures are

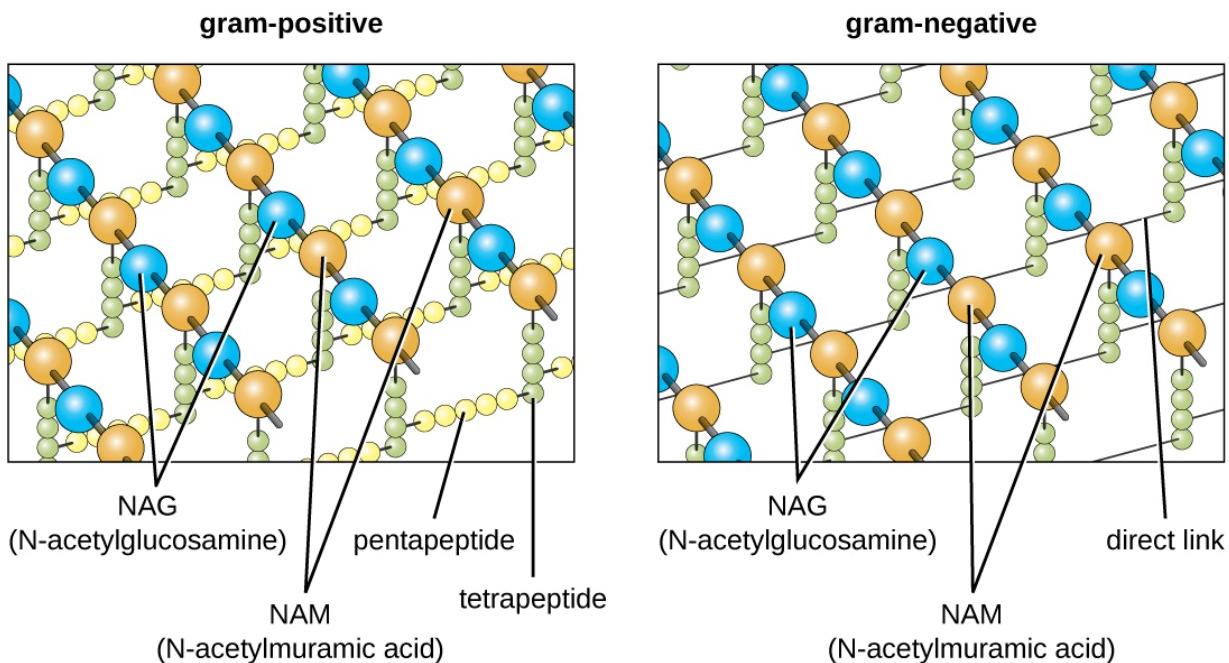
called thylakoids; in photosynthetic bacteria, they are called chromatophores, lamellae, or chlorosomes.

Cell Wall

The primary function of the cell wall is to protect the cell from harsh conditions in the outside environment. When present, there are notable similarities and differences among the cell walls of archaea, bacteria, and eukaryotes.

The major component of bacterial cell walls is called **peptidoglycan** (or murein); it is only found in bacteria. Structurally, peptidoglycan resembles a layer of meshwork or fabric ([\[link\]](#)). Each layer is composed of long chains of alternating molecules of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM). The structure of the long chains has significant two-dimensional tensile strength due to the formation of peptide bridges that connect NAG and NAM within each peptidoglycan layer. In gram-negative bacteria, tetrapeptide chains extending from each NAM unit are directly cross-linked, whereas in gram-positive bacteria, these tetrapeptide chains are linked by pentaglycine cross-bridges. Peptidoglycan subunits are made inside of the bacterial cell and then exported and assembled in layers, giving the cell its shape.

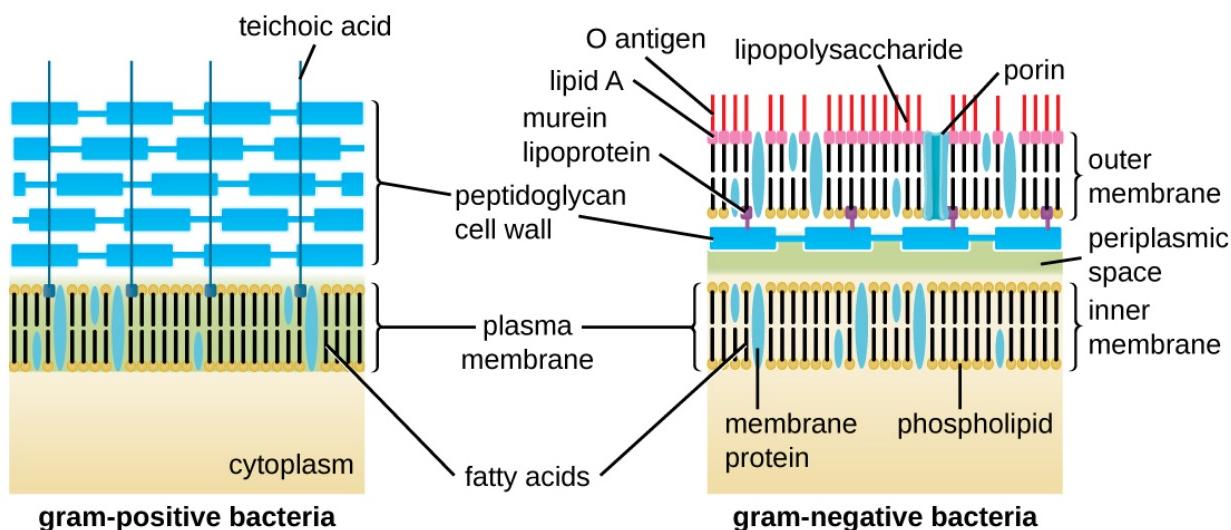
Since peptidoglycan is unique to bacteria, many antibiotic drugs are designed to interfere with peptidoglycan synthesis, weakening the cell wall and making bacterial cells more susceptible to the effects of osmotic pressure (see [Mechanisms of Antibacterial Drugs](#)). In addition, certain cells of the human immune system are able “recognize” bacterial pathogens by detecting peptidoglycan on the surface of a bacterial cell; these cells then engulf and destroy the bacterial cell, using enzymes such as lysozyme, which breaks down and digests the peptidoglycan in their cell walls (see [Pathogen Recognition and Phagocytosis](#)).



Peptidoglycan is composed of polymers of alternating NAM and NAG subunits, which are cross-linked by peptide bridges linking NAM subunits from various glycan chains. This provides the cell wall with tensile strength in two dimensions.

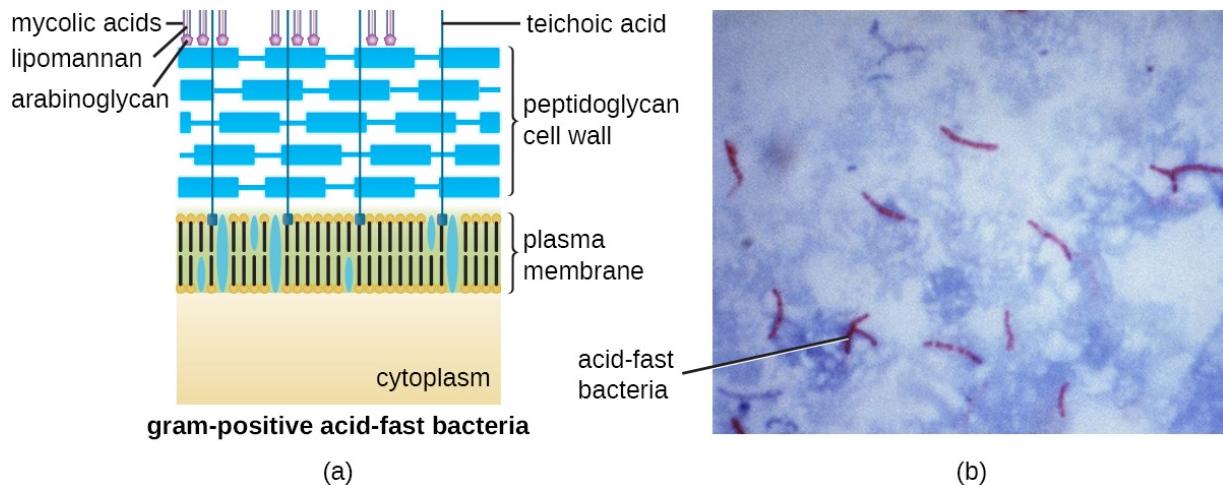
The Gram staining protocol (see [Staining Microscopic Specimens](#)) is used to differentiate two common types of cell wall structures ([\[link\]](#)). Gram-positive cells have a cell wall consisting of many layers of peptidoglycan totaling 30–100 nm in thickness. These peptidoglycan layers are commonly embedded with teichoic acids (TAs), carbohydrate chains that extend through and beyond the peptidoglycan layer.[\[footnote\]](#) TA is thought to stabilize peptidoglycan by increasing its rigidity. TA also plays a role in the ability of pathogenic gram-positive bacteria such as *Streptococcus* to bind to certain proteins on the surface of host cells, enhancing their ability to cause infection. In addition to peptidoglycan and TAs, bacteria of the family Mycobacteriaceae have an external layer of waxy **mycolic acids** in their cell wall; as described in [Staining Microscopic Specimens](#), these bacteria are referred to as acid-fast, since acid-fast stains must be used to penetrate the mycolic acid layer for purposes of microscopy ([\[link\]](#)).

T.J. Silhavy, D. Kahne, S. Walker. "The Bacterial Cell Envelope." *Cold Spring Harbor Perspectives in Biology* 2 no. 5 (2010):a000414.



Bacteria contain two common cell wall structural types. Gram-positive cell walls are structurally simple, containing a thick layer of peptidoglycan with embedded teichoic acid external to the plasma membrane.[\[footnote\]](#) Gram-negative cell walls are structurally more complex, containing three layers: the inner membrane, a thin layer of peptidoglycan, and an outer membrane containing lipopolysaccharide.
(credit: modification of work by "Franciscosp2"/Wikimedia Commons)

B. Zuber et al. "Granular Layer in the Periplasmic Space of Gram-Positive Bacteria and Fine Structures of *Enterococcus gallinarum* and *Streptococcus gordonii* Septa Revealed by Cryo-Electron Microscopy of Vitreous Sections." *Journal of Bacteriology* 188 no. 18 (2006):6652–6660

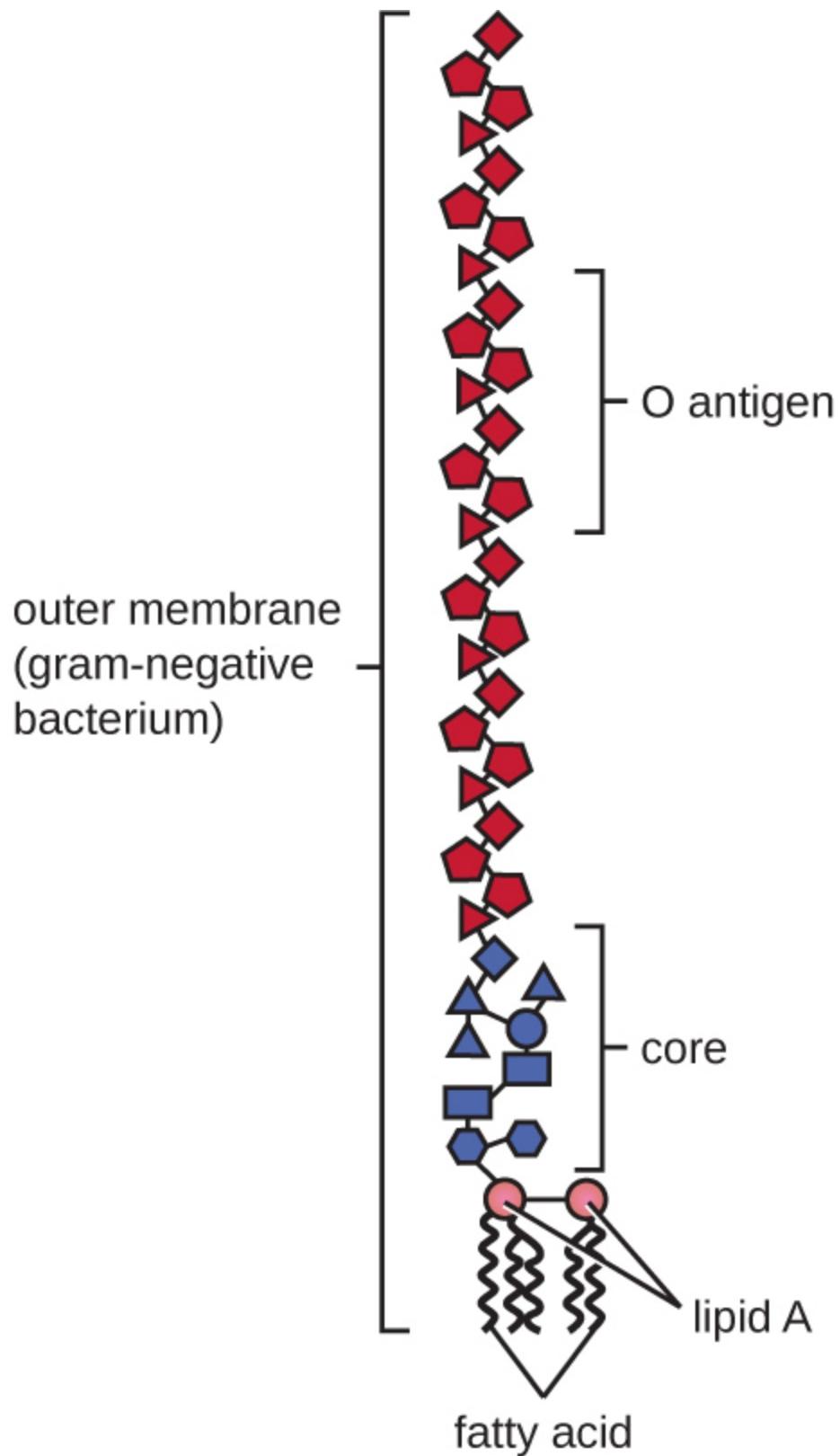


(a) Some gram-positive bacteria, including members of the Mycobacteriaceae, produce waxy mycolic acids found exterior to their structurally-distinct peptidoglycan. (b) The acid-fast staining protocol detects the presence of cell walls that are rich in mycolic acid. Acid-fast cells are stained red by carbolfuscin. (credit a: modification of work by “Franciscosp2”/Wikimedia Commons; credit b: modification of work by Centers for Disease Control and Prevention)

Gram-negative cells have a much thinner layer of peptidoglycan (no more than about 4 nm thick[\[footnote\]](#)) than gram-positive cells, and the overall structure of their cell envelope is more complex. In gram-negative cells, a gel-like matrix occupies the **periplasmic space** between the cell wall and the plasma membrane, and there is a second lipid bilayer called the **outer membrane**, which is external to the peptidoglycan layer ([\[link\]](#)). This outer membrane is attached to the peptidoglycan by murein lipoprotein. The outer leaflet of the outer membrane contains the molecule **lipopolysaccharide (LPS)**, which functions as an endotoxin in infections involving gram-negative bacteria, contributing to symptoms such as fever, hemorrhaging, and septic shock. Each LPS molecule is composed of Lipid A, a core polysaccharide, and an O side chain that is composed of sugar-like molecules that comprise the external face of the LPS ([\[link\]](#)). The composition of the O side chain varies between different species and strains of bacteria. Parts of the O side chain called antigens can be detected using serological or immunological tests to identify specific pathogenic strains

like *Escherichia coli* O157:H7, a deadly strain of bacteria that causes bloody diarrhea and kidney failure.

L. Gana, S. Chena, G.J. Jensen. "Molecular Organization of Gram-Negative Peptidoglycan." *Proceedings of the National Academy of Sciences of the United States of America* 105 no. 48 (2008):18953–18957.



The outer membrane of a gram-negative bacterial

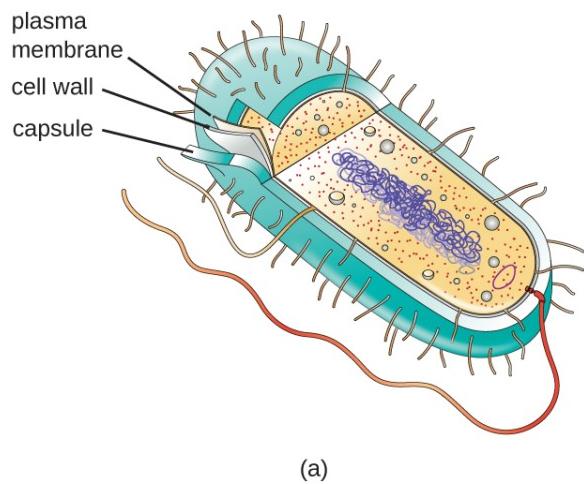
cell contains lipopolysaccharide (LPS), a toxin composed of Lipid A embedded in the outer membrane, a core polysaccharide, and the O side chain.

Archaeal cell wall structure differs from that of bacteria in several significant ways. First, archaeal cell walls do not contain peptidoglycan; instead, they contain a similar polymer called pseudopeptidoglycan (pseudomurein) in which NAM is replaced with a different subunit. Other archaea may have a layer of glycoproteins or polysaccharides that serves as the cell wall instead of pseudopeptidoglycan. Last, as is the case with some bacterial species, there are a few archaea that appear to lack cell walls entirely.

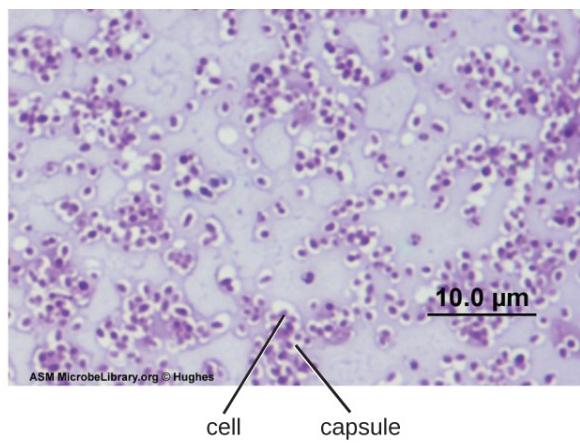
Glycocalyces and S-Layers

Although most prokaryotic cells have cell walls, some may have additional cell envelope structures exterior to the cell wall, such as glycocalyces and S-layers. A **glycocalyx** is a sugar coat, of which there are two important types: capsules and slime layers. A **capsule** is an organized layer located outside of the cell wall and usually composed of polysaccharides or proteins ([\[link\]](#)). A **slime layer** is a less tightly organized layer that is only loosely attached to the cell wall and can be more easily washed off. Slime layers may be composed of polysaccharides, glycoproteins, or glycolipids.

Glycocalyces allows cells to adhere to surfaces, aiding in the formation of biofilms (colonies of microbes that form in layers on surfaces). In nature, most microbes live in mixed communities within biofilms, partly because the biofilm affords them some level of protection. Biofilms generally hold water like a sponge, preventing desiccation. They also protect cells from predation and hinder the action of antibiotics and disinfectants. All of these properties are advantageous to the microbes living in a biofilm, but they present challenges in a clinical setting, where the goal is often to eliminate microbes.



(a)



(b)

(a) Capsules are a type of glycocalyx composed of an organized layer of polysaccharides. (b) A capsule stain of *Pseudomonas aeruginosa*, a bacterial pathogen capable of causing many different types of infections in humans. (credit b: modification of work by American Society for Microbiology)

The ability to produce a capsule can contribute to a microbe's pathogenicity (ability to cause disease) because the capsule can make it more difficult for phagocytic cells (such as white blood cells) to engulf and kill the microorganism. *Streptococcus pneumoniae*, for example, produces a capsule that is well known to aid in this bacterium's pathogenicity. As explained in [Staining Microscopic specimens](#), capsules are difficult to stain for microscopy; negative staining techniques are typically used.

An **S-layer** is another type of cell envelope structure; it is composed of a mixture of structural proteins and glycoproteins. In bacteria, S-layers are found outside the cell wall, but in some archaea, the S-layer serves as the cell wall. The exact function of S-layers is not entirely understood, and they are difficult to study; but available evidence suggests that they may play a variety of functions in different prokaryotic cells, such as helping the cell withstand osmotic pressure and, for certain pathogens, interacting with the host immune system.

Filamentous Appendages

Many bacterial cells have protein appendages embedded within their cell envelopes that extend outward, allowing interaction with the environment. These appendages can attach to other surfaces, transfer DNA, or provide movement. Filamentous appendages include fimbriae, pili, and flagella.

Fimbriae and Pili

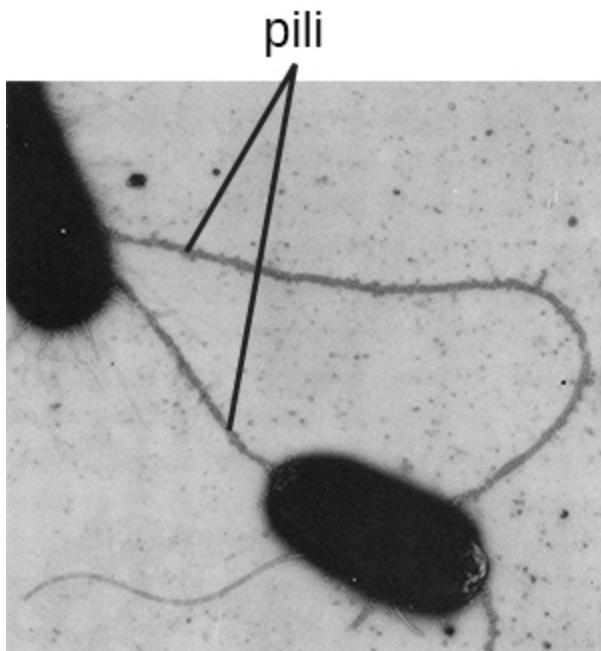
Fimbriae and pili are structurally similar and, because differentiation between the two is problematic, these terms are often used interchangeably.

[footnote] [footnote] The term **fimbriae** commonly refers to short bristle-like proteins projecting from the cell surface by the hundreds. Fimbriae enable a cell to attach to surfaces and to other cells. For pathogenic bacteria, adherence to host cells is important for colonization, infectivity, and virulence. Adherence to surfaces is also important in biofilm formation.

J.A. Garnetta et al. “Structural Insights Into the Biogenesis and Biofilm Formation by the *Escherichia coli* Common Pilus.” *Proceedings of the National Academy of Sciences of the United States of America* 109 no. 10 (2012):3950–3955.

T. Proft, E.N. Baker. “Pili in Gram-Negative and Gram-Positive Bacteria—Structure, Assembly and Their Role in Disease.” *Cellular and Molecular Life Sciences* 66 (2009):613.

The term **pili** (singular: pilus) commonly refers to longer, less numerous protein appendages that aid in attachment to surfaces ([\[link\]](#)). A specific type of pilus, called the **F pilus** or **sex pilus**, is important in the transfer of DNA between bacterial cells, which occurs between members of the same generation when two cells physically transfer or exchange parts of their respective genomes (see [How Asexual Prokaryotes Achieve Genetic Diversity](#)).



Bacteria may produce two different types of protein appendages that aid in surface attachment. Fimbriae typically are more numerous and shorter, whereas pili (shown here) are longer and less numerous per cell. (credit: modification of work by American Society for Microbiology)

Note:

Group A Strep

Before the structure and function of the various components of the bacterial cell envelope were well understood, scientists were already using cell envelope characteristics to classify bacteria. In 1933, Rebecca Lancefield proposed a method for serotyping various β -hemolytic strains of *Streptococcus* species using an agglutination assay, a technique using

the clumping of bacteria to detect specific cell-surface antigens. In doing so, Lancefield discovered that one group of *S. pyogenes*, found in Group A, was associated with a variety of human diseases. She determined that various strains of Group A strep could be distinguished from each other based on variations in specific cell surface proteins that she named M proteins.

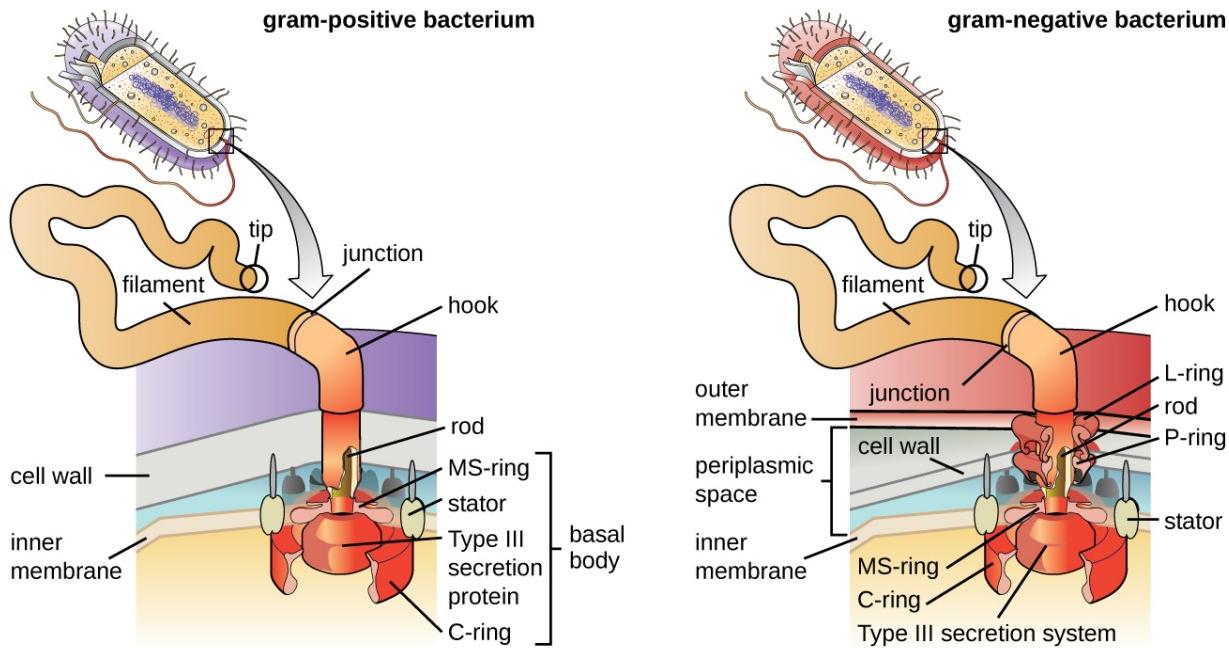
Today, more than 80 different strains of Group A strep have been identified based on M proteins. Various strains of Group A strep are associated with a wide variety of human infections, including streptococcal pharyngitis (strep throat), impetigo, toxic shock syndrome, scarlet fever, rheumatic fever, and necrotizing fasciitis. The M protein is an important virulence factor for Group A strep, helping these strains evade the immune system. Changes in M proteins appear to alter the infectivity of a particular strain of Group A strep.

Flagella

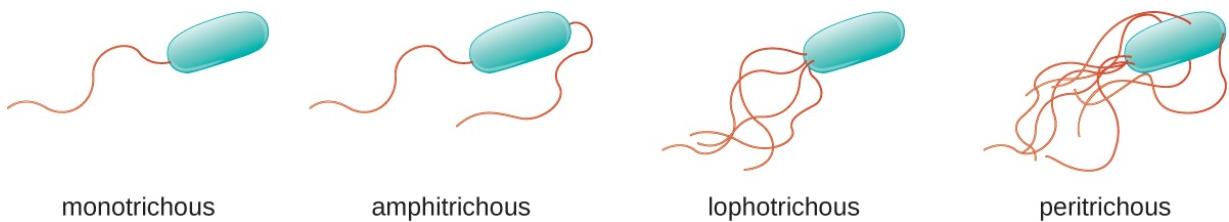
Flagella are structures used by cells to move in aqueous environments. Bacterial flagella act like propellers. They are stiff spiral filaments composed of flagellin protein subunits that extend outward from the cell and spin in solution. The **basal body** is the motor for the flagellum and is embedded in the plasma membrane ([\[link\]](#)). A hook region connects the basal body to the filament. Gram-positive and gram-negative bacteria have different basal body configurations due to differences in cell wall structure.

Different types of motile bacteria exhibit different arrangements of flagella ([\[link\]](#)). A bacterium with a singular flagellum, typically located at one end of the cell (polar), is said to have a **monotrichous** flagellum. An example of a monotrichously flagellated bacterial pathogen is *Vibrio cholerae*, the gram-negative bacterium that causes cholera. Cells with **amphitrichous** flagella have a flagellum or tufts of flagella at each end. An example is *Spirillum minor*, the cause of spirillary (Asian) rat-bite fever or sodoku. Cells with **lophotrichous** flagella have a tuft at one end of the cell. The gram-negative bacillus *Pseudomonas aeruginosa*, an opportunistic pathogen known for causing many infections, including “swimmer’s ear” and burn wound infections, has lophotrichous flagella. Flagella that cover

the entire surface of a bacterial cell are called **peritrichous** flagella. The gram-negative bacterium *E. coli* shows a peritrichous arrangement of flagella.



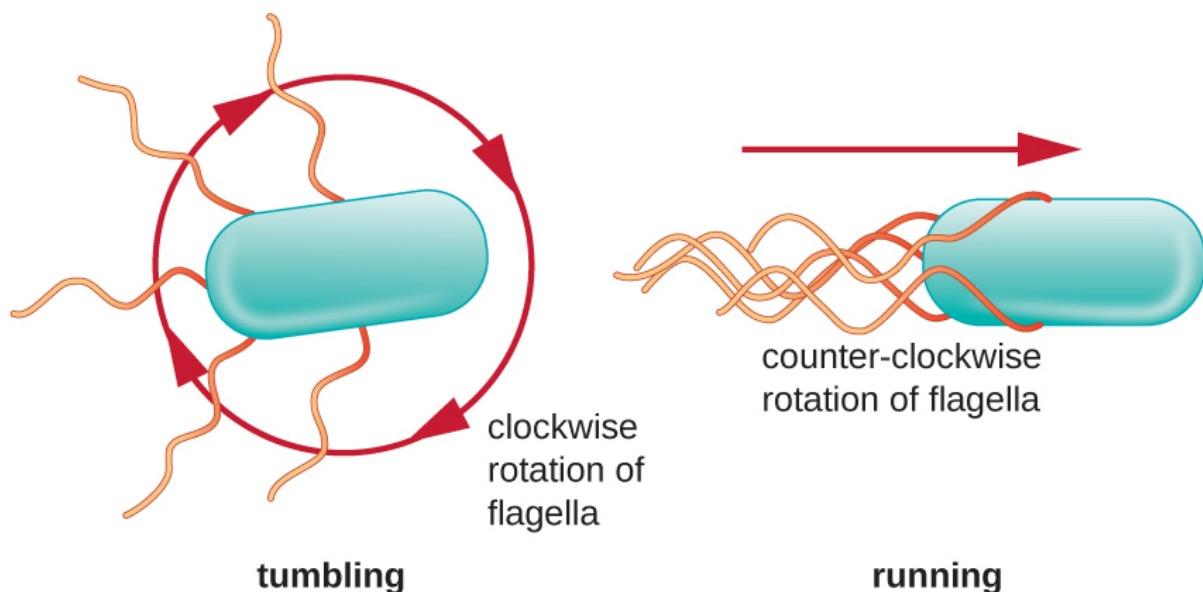
The basic structure of a bacterial flagellum consists of a basal body, hook, and filament. The basal body composition and arrangement differ between gram-positive and gram-negative bacteria. (credit: modification of work by “LadyofHats”/Mariana Ruiz Villareal)



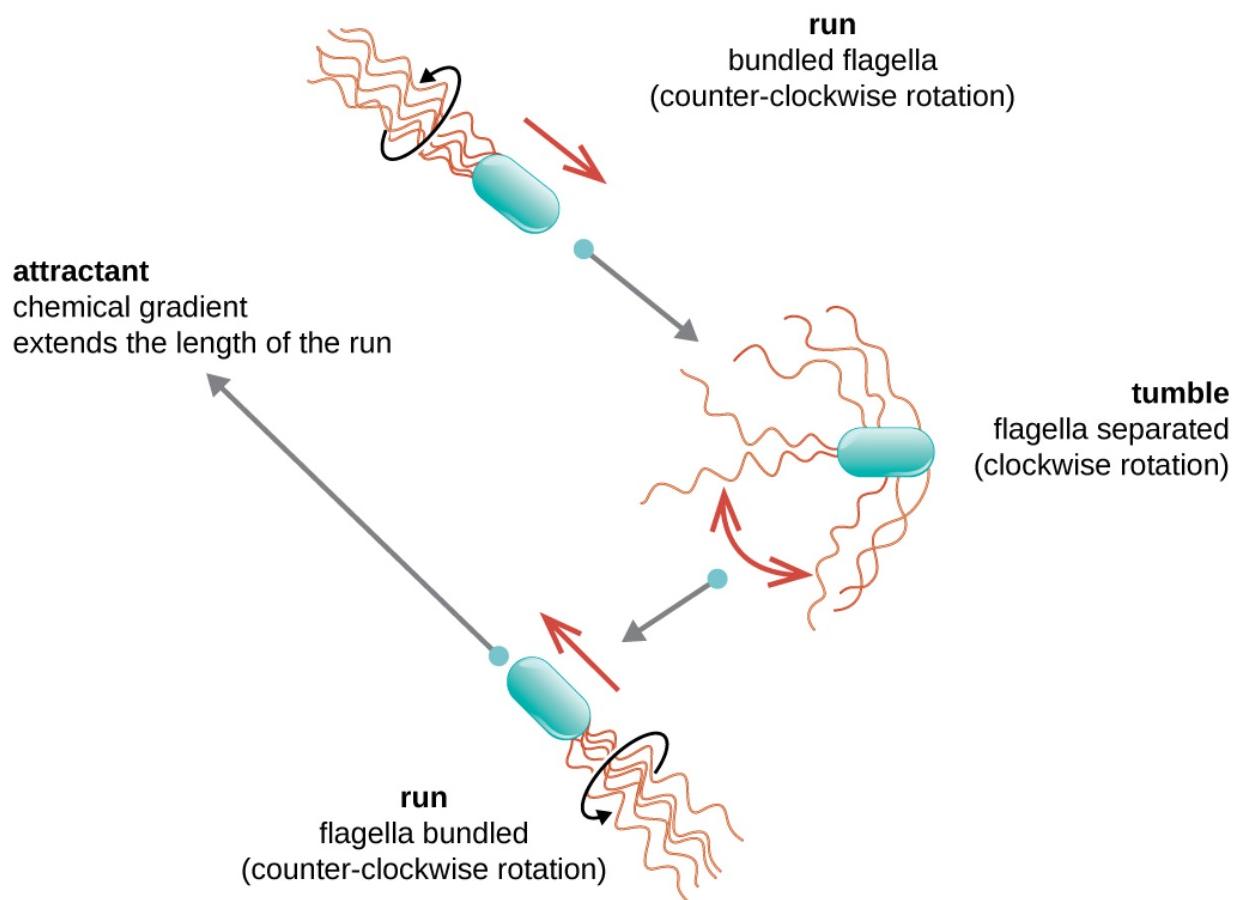
Flagellated bacteria may exhibit multiple arrangements of their flagella. Common arrangements include monotrichous, amphitrichous,

lophotrichous, or peritrichous.

Directional movement depends on the configuration of the flagella. Bacteria can move in response to a variety of environmental signals, including light (**phototaxis**), magnetic fields (**magnetotaxis**) using magnetosomes, and, most commonly, chemical gradients (**chemotaxis**). Purposeful movement toward a chemical attractant, like a food source, or away from a repellent, like a poisonous chemical, is achieved by increasing the length of **runs** and decreasing the length of **tumbles**. When running, flagella rotate in a counterclockwise direction, allowing the bacterial cell to move forward. In a peritrichous bacterium, the flagella are all bundled together in a very streamlined way ([link]), allowing for efficient movement. When tumbling, flagella are splayed out while rotating in a clockwise direction, creating a looping motion and preventing meaningful forward movement but reorienting the cell toward the direction of the attractant. When an attractant exists, runs and tumbles still occur; however, the length of runs is longer, while the length of the tumbles is reduced, allowing overall movement toward the higher concentration of the attractant. When no chemical gradient exists, the lengths of runs and tumbles are more equal, and overall movement is more random ([link]).



Bacteria achieve directional movement by changing the rotation of their flagella. In a cell with peritrichous flagella, the flagella bundle when they rotate in a counterclockwise direction, resulting in a run. However, when the flagella rotate in a clockwise direction, the flagella are no longer bundled, resulting in tumbles.



Without a chemical gradient, flagellar rotation cycles between counterclockwise (run) and clockwise (tumble) with no overall directional movement. However, when a chemical gradient of an attractant exists, the length of runs is extended, while the length of tumbles is decreased. This leads to chemotaxis: an overall directional movement toward the higher concentration of the attractant.

Note:

- What is the peptidoglycan layer and how does it differ between gram-positive and gram-negative bacteria?
- Compare and contrast monotrichous, amphitrichous, lophotrichous, and peritrichous flagella.

Key Concepts and Summary

- Prokaryotic cells differ from eukaryotic cells in that their genetic material is contained in a **nucleoid** rather than a membrane-bound nucleus. In addition, prokaryotic cells generally lack membrane-bound organelles.
- Prokaryotic cells of the same species typically share a similar **cell morphology** and **cellular arrangement**.
- Most prokaryotic cells have a **cell wall** that helps the organism maintain cellular morphology and protects it against changes in osmotic pressure.
- Outside of the nucleoid, prokaryotic cells may contain extrachromosomal DNA in **plasmids**.
- Prokaryotic **ribosomes** that are found in the cytoplasm have a size of 70S.
- Some prokaryotic cells have **inclusions** that store nutrients or chemicals for other uses.
- Some prokaryotic cells are able to form **endospores** through **sporulation** to survive in a dormant state when conditions are unfavorable. Endospores can **germinate**, transforming back into **vegetative cells** when conditions improve.
- In prokaryotic cells, the **cell envelope** includes a **plasma membrane** and usually a cell wall.

- Bacterial membranes are composed of phospholipids with integral or peripheral proteins. The fatty acid components of these phospholipids are ester-linked and are often used to identify specific types of bacteria. The proteins serve a variety of functions, including transport, cell-to-cell communication, and sensing environmental conditions. Archaeal membranes are distinct in that they are composed of fatty acids that are ether-linked to phospholipids.
- Some molecules can move across the bacterial membrane by simple diffusion, but most large molecules must be actively transported through membrane structures using cellular energy.
- Prokaryotic cell walls may be composed of **peptidoglycan** (bacteria) or **pseudopeptidoglycan** (archaea).
- Gram-positive bacterial cells are characterized by a thick **peptidoglycan** layer, whereas gram-negative bacterial cells are characterized by a thin peptidoglycan layer surrounded by an outer membrane.
- Some prokaryotic cells produce **glycocalyx** coatings, such as **capsules** and **slime layers**, that aid in attachment to surfaces and/or evasion of the host immune system.
- Some prokaryotic cells have **fimbriae** or **pili**, filamentous appendages that aid in attachment to surfaces. Pili are also used in the transfer of genetic material between cells.
- Some prokaryotic cells use one or more **flagella** to move through water. **Peritrichous** bacteria, which have numerous flagella, use **runs** and **tumbles** to move purposefully in the direction of a chemical attractant.

Critical Thinking

Exercise:

Problem:

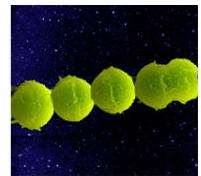
Which of the following slides is a good example of staphylococci?



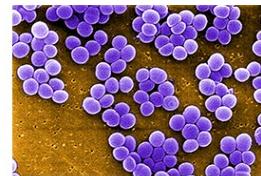
A



B



C



D

(credit a: modification of work by U.S. Department of Agriculture; credit b: modification of work by Centers for Disease Control and Prevention; credit c: modification of work by NIAID)

Exercise:

Problem:

Provide some examples of bacterial structures that might be used as antibiotic targets and explain why.

Exercise:

Problem:

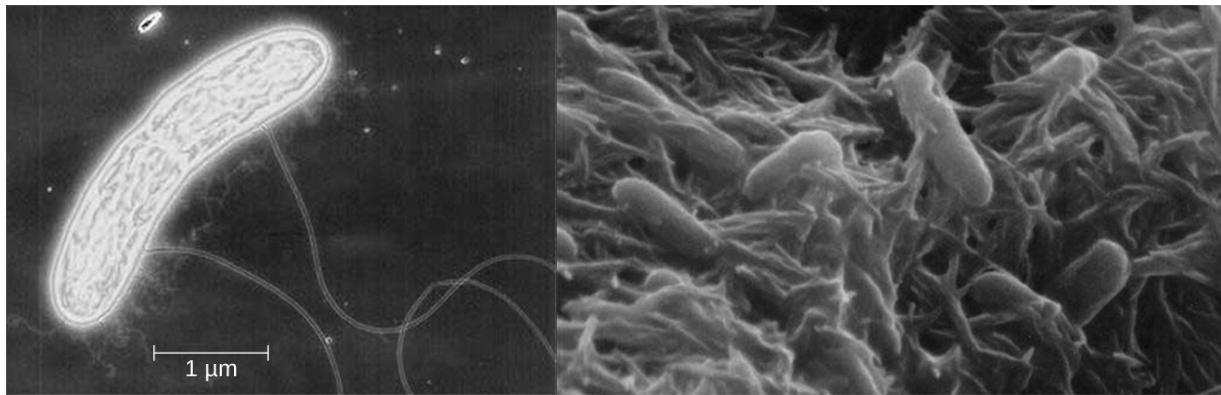
The causative agent of botulism, a deadly form of food poisoning, is an endospore-forming bacterium called *Clostridium botulinum*. Why might it be difficult to kill this bacterium in contaminated food?

Prokaryotic Cells - Introduction

class="introduction"

The bacterium *Shewanella* lives in the deep sea, where there is little oxygen diffused in the water. It is able to survive in this harsh environment by attaching to the sea floor and using long appendages, called "nanocables," to sense oxygen.

(credit a: modification of work by NASA; credit b: modification of work by Liza Gross)



Scientists have studied prokaryotes for centuries, but it wasn't until 1966 that scientist Thomas Brock (1926–) discovered that certain bacteria can live in boiling water. This led many to wonder whether prokaryotes may also live in other extreme environments, such as at the bottom of the ocean, at high altitudes, or inside volcanoes, or even on other planets.

Prokaryotes have an important role in changing, shaping, and sustaining the entire biosphere. They can produce proteins and other substances used by molecular biologists in basic research and in medicine and industry. For example, the bacterium *Shewanella* lives in the deep sea, where oxygen is scarce. It grows long appendages, which have special sensors used to seek the limited oxygen in its environment. It can also digest toxic waste and generate electricity. Other species of prokaryotes can produce more oxygen than the entire Amazon rainforest, while still others supply plants, animals, and humans with usable forms of nitrogen; and inhabit our body, protecting us from harmful microorganisms and producing some vitally important substances. This chapter will examine the diversity, structure, and function of prokaryotes.

Prokaryote Habitats, Relationships, and Microbiomes

LEARNING OBJECTIVES

- Identify and describe unique examples of prokaryotes in various habitats on earth
- Identify and describe symbiotic relationships
- Compare normal/commensal/resident microbiota to transient microbiota
- Explain how prokaryotes are classified

All living organisms are classified into three domains of life: Archaea, Bacteria, and Eukarya. In this chapter, we will focus on the domains Archaea and Bacteria. Archaea and bacteria are unicellular prokaryotic organisms. Unlike eukaryotes, they have no nuclei or any other membrane-bound organelles.

Prokaryote Habitats and Functions

Prokaryotes are ubiquitous. They can be found everywhere on our planet, even in hot springs, in the Antarctic ice shield, and under extreme pressure two miles under water. One bacterium, *Paracoccus denitrificans*, has even been shown to survive when scientists removed it from its native environment (soil) and used a centrifuge to subject it to forces of gravity as strong as those found on the surface of Jupiter.

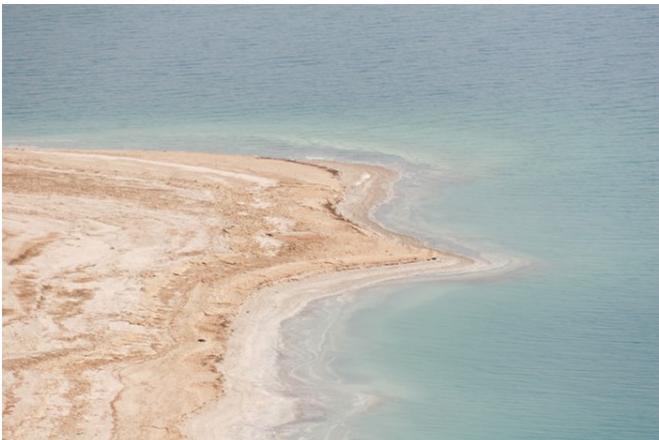
Prokaryotes also are abundant on and within the human body. According to a report by National Institutes of Health, prokaryotes, especially bacteria, outnumber human cells 10:1.[\[footnote\]](#) More recent studies suggest the

ratio could be closer to 1:1, but even that ratio means that there are a great number of bacteria within the human body.[\[footnote\]](#) Bacteria thrive in the human mouth, nasal cavity, throat, ears, gastrointestinal tract, and vagina. Large colonies of bacteria can be found on healthy human skin, especially in moist areas (armpits, navel, and areas behind ears). However, even drier areas of the skin are not free from bacteria.

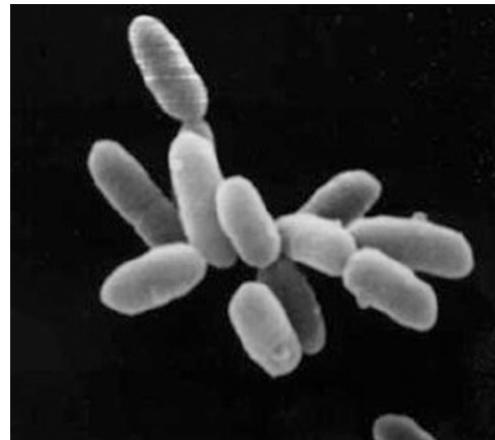
Medical Press. "Mouth Bacteria Can Change Their Diet, Supercomputers Reveal." August 12, 2014. <http://medicalxpress.com/news/2014-08-mouth-bacteria-diet-supercomputers-reveal.html>. Accessed February 24, 2015.

A. Abbott. "Scientists Bust Myth That Our Bodies Have More Bacteria Than Human Cells: Decades-Old Assumption about Microbiota Revisited." *Nature*. <http://www.nature.com/news/scientists-bust-myth-that-our-bodies-have-more-bacteria-than-human-cells-1.19136>. Accessed June 3, 2016.

The existence of prokaryotes is very important for the stability and thriving of ecosystems. For example, they are a necessary part of soil formation and stabilization processes through the breakdown of organic matter and development of biofilms. One gram of soil contains up to 10 billion microorganisms (most of them prokaryotic) belonging to about 1,000 species. Many species of bacteria use substances released from plant roots, such as acids and carbohydrates, as nutrients. The bacteria metabolize these plant substances and release the products of bacterial metabolism back to the soil, forming humus and thus increasing the soil's fertility. In salty lakes such as the Dead Sea ([\[link\]](#)), salt-loving halobacteria decompose dead brine shrimp and nourish young brine shrimp and flies with the products of bacterial metabolism.



(a)



(b)

(a) Some prokaryotes, called halophiles, can thrive in extremely salty environments such as the Dead Sea, pictured here. (b) The archaeon *Halobacterium salinarum*, shown here in an electron micrograph, is a halophile that lives in the Dead Sea. (credit a: modification of work by Jullen Menichini; credit b: modification of work by NASA)

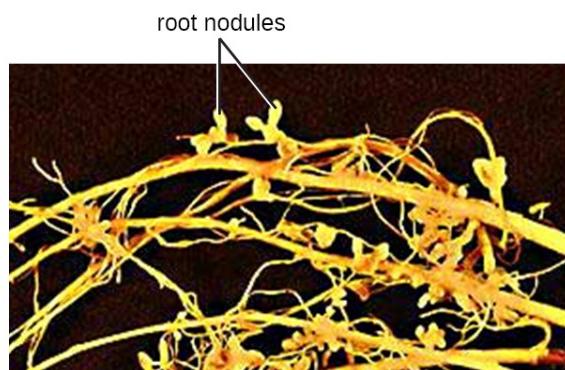
In addition to living in the ground and the water, prokaryotic microorganisms are abundant in the air, even high in the atmosphere. There may be up to 2,000 different kinds of bacteria in the air, similar to their diversity in the soil.

Prokaryotes can be found everywhere on earth because they are extremely resilient and adaptable. They are often metabolically flexible, which means that they might easily switch from one energy source to another, depending on the availability of the sources, or from one metabolic pathway to another. For example, certain prokaryotic cyanobacteria can switch from a conventional type of lipid metabolism, which includes production of fatty aldehydes, to a different type of lipid metabolism that generates biofuel, such as fatty acids and wax esters. Groundwater bacteria store complex high-energy carbohydrates when grown in pure groundwater, but they metabolize these molecules when the groundwater is enriched with phosphates. Some bacteria get their energy by reducing sulfates into

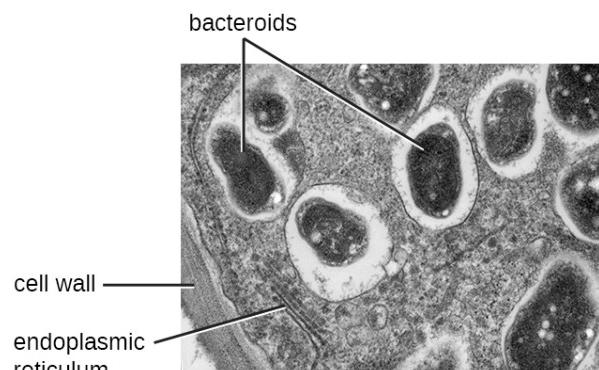
sulfides, but can switch to a different metabolic pathway when necessary, producing acids and free hydrogen ions.

Prokaryotes perform functions vital to life on earth by capturing (or “fixing”) and recycling elements like carbon and nitrogen. Organisms such as animals require organic carbon to grow, but, unlike prokaryotes, they are unable to use inorganic carbon sources like carbon dioxide. Thus, animals rely on prokaryotes to convert carbon dioxide into organic carbon products that they can use. This process of converting carbon dioxide to organic carbon products is called carbon fixation.

Plants and animals also rely heavily on prokaryotes for nitrogen fixation, the conversion of atmospheric nitrogen into ammonia, a compound that some plants can use to form many different biomolecules necessary to their survival. Bacteria in the genus *Rhizobium*, for example, are nitrogen-fixing bacteria; they live in the roots of legume plants such as clover, alfalfa, and peas ([\[link\]](#)). Ammonia produced by *Rhizobium* helps these plants to survive by enabling them to make building blocks of nucleic acids. In turn, these plants may be eaten by animals—sustaining their growth and survival—or they may die, in which case the products of nitrogen fixation will enrich the soil and be used by other plants.



(a)



(b)

- (a) Nitrogen-fixing bacteria such as *Rhizobium* live in the root nodules of legumes such as clover. (b) This micrograph of the root nodule shows bacteroids (bacterium-like cells or modified bacterial cells)

within the plant cells. The bacteroids are visible as darker ovals within the larger plant cell. (credit a: modification of work by USDA)

Another positive function of prokaryotes is in cleaning up the environment. Recently, some researchers focused on the diversity and functions of prokaryotes in manmade environments. They found that some bacteria play a unique role in degrading toxic chemicals that pollute water and soil.

[footnote]

A.M. Kravetz “Unique Bacteria Fights Man-Made Chemical Waste.” 2012. <http://www.livescience.com/25181-bacteria-strain-cleans-up-toxins-nsf-bts.html>. Accessed March 9, 2015.

Despite all of the positive and helpful roles prokaryotes play, some are human pathogens that may cause illness or infection when they enter the body. In addition, some bacteria can contaminate food, causing spoilage or foodborne illness, which makes them subjects of concern in food preparation and safety. Less than 1% of prokaryotes (all of them bacteria) are thought to be human pathogens, but collectively these species are responsible for a large number of the diseases that afflict humans.

Besides pathogens, which have a direct impact on human health, prokaryotes also affect humans in many indirect ways. For example, prokaryotes are now thought to be key players in the processes of climate change. In recent years, as temperatures in the earth’s polar regions have risen, soil that was formerly frozen year-round (permafrost) has begun to thaw. Carbon trapped in the permafrost is gradually released and metabolized by prokaryotes. This produces massive amounts of carbon dioxide and methane, greenhouse gases that escape into the atmosphere and contribute to the greenhouse effect.

Note:

- In what types of environments can prokaryotes be found?
- Name some ways that plants and animals rely on prokaryotes.

Symbiotic Relationships

As we have learned, prokaryotic microorganisms can associate with plants and animals. Often, this association results in unique relationships between organisms. For example, bacteria living on the roots or leaves of a plant get nutrients from the plant and, in return, produce substances that protect the plant from pathogens. On the other hand, some bacteria are plant pathogens that use mechanisms of infection similar to bacterial pathogens of animals and humans.

Prokaryotes live in a **community**, or a group of interacting populations of organisms. A population is a group of individual organisms belonging to the same biological species and limited to a certain geographic area.

Populations can have **cooperative interactions**, which benefit the populations, or **competitive interactions**, in which one population competes with another for resources. The study of these interactions between populations is called **microbial ecology**.

Any interaction between different species within a community is called **symbiosis**. Such interactions fall along a continuum between opposition and cooperation. Interactions in a symbiotic relationship may be beneficial or harmful, or have no effect on one or both of the species involved. [\[link\]](#) summarizes the main types of symbiotic interactions among prokaryotes.

Types of Symbiotic Relationships

Type	Population A	Population B
Mutualism	Benefitted	Benefitted
Amensalism	Harmed	Unaffected

Types of Symbiotic Relationships

Type	Population A	Population B
Commensalism	Benefitted	Unaffected
Neutralism	Unaffected	Unaffected
Parasitism	Benefitted	Harmed

When two species benefit from each other, the symbiosis is called **mutualism** (or syntropy, or crossfeeding). For example, humans have a mutualistic relationship with the bacterium *Bacteroides thetaiotetraiotamicron*, which lives in the intestinal tract. *B. thetaiotetraiotamicron* digests complex polysaccharide plant materials that human digestive enzymes cannot break down, converting them into monosaccharides that can be absorbed by human cells. Humans also have a mutualistic relationship with certain strains of *Escherichia coli*, another bacterium found in the gut. *E. coli* relies on intestinal contents for nutrients, and humans derive certain vitamins from *E. coli*, particularly vitamin K, which is required for the formation of blood clotting factors. (This is only true for some strains of *E. coli*, however. Other strains are pathogenic and do not have a mutualistic relationship with humans.)

A type of symbiosis in which one population harms another but remains unaffected itself is called **amensalism**. In the case of bacteria, some amensalist species produce bactericidal substances that kill other species of bacteria. For example, the bacterium *Lucilia sericata* produces a protein that destroys *Staphylococcus aureus*, a bacterium commonly found on the surface of the human skin. Too much handwashing can affect this relationship and lead to *S. aureus* diseases and transmission.

In another type of symbiosis, called **commensalism**, one organism benefits while the other is unaffected. This occurs when the bacterium *Staphylococcus epidermidis* uses the dead cells of the human skin as nutrients. Billions of these bacteria live on our skin, but in most cases

(especially when our immune system is healthy), we do not react to them in any way.

If neither of the symbiotic organisms is affected in any way, we call this type of symbiosis **neutralism**. An example of neutralism is the coexistence of metabolically active (vegetating) bacteria and endospores (dormant, metabolically passive bacteria). For example, the bacterium *Bacillus anthracis* typically forms endospores in soil when conditions are unfavorable. If the soil is warmed and enriched with nutrients, some endospores germinate and remain in symbiosis with other endospores that have not germinated.

A type of symbiosis in which one organism benefits while harming the other is called **parasitism**. The relationship between humans and many pathogenic prokaryotes can be characterized as parasitic because these organisms invade the body, producing toxic substances or infectious diseases that cause harm. Diseases such as tetanus, diphtheria, pertussis, tuberculosis, and leprosy all arise from interactions between bacteria and humans.

Scientists have coined the term **microbiome** to refer to all prokaryotic and eukaryotic microorganisms that are associated with a certain organism. Within the human microbiome, there are **resident microbiota** and **transient microbiota**. The resident microbiota consists of microorganisms that constantly live in or on our bodies. The term transient microbiota refers to microorganisms that are only temporarily found in the human body, and these may include pathogenic microorganisms. Hygiene and diet can alter both the resident and transient microbiota.

The resident microbiota is amazingly diverse, not only in terms of the variety of species but also in terms of the preference of different microorganisms for different areas of the human body. For example, in the human mouth, there are thousands of commensal or mutualistic species of bacteria. Some of these bacteria prefer to inhabit the surface of the tongue, whereas others prefer the internal surface of the cheeks, and yet others prefer the front or back teeth or gums. The inner surface of the cheek has the least diverse microbiota because of its exposure to oxygen. By contrast, the crypts of the tongue and the spaces between teeth are two sites with

limited oxygen exposure, so these sites have more diverse microbiota, including bacteria living in the absence of oxygen (e.g., *Bacteroides*, *Fusobacterium*). Differences in the oral microbiota between randomly chosen human individuals are also significant. Studies have shown, for example, that the prevalence of such bacteria as *Streptococcus*, *Haemophilus*, *Neisseria*, and others was dramatically different when compared between individuals.[\[footnote\]](#)

E.M. Bik et al. “Bacterial Diversity in the Oral Cavity of 10 Healthy Individuals.” *The ISME Journal* 4 no. 8 (2010):962–974.

There are also significant differences between the microbiota of different sites of the same human body. The inner surface of the cheek has a predominance of *Streptococcus*, whereas in the throat, the palatine tonsil, and saliva, there are two to three times fewer *Streptococcus*, and several times more *Fusobacterium*. In the plaque removed from gums, the predominant bacteria belong to the genus *Fusobacterium*. However, in the intestine, both *Streptococcus* and *Fusobacterium* disappear, and the genus *Bacteroides* becomes predominant.

Not only can the microbiota vary from one body site to another, the microbiome can also change over time within the same individual. Humans acquire their first inoculations of normal flora during natural birth and shortly after birth. Before birth, there is a rapid increase in the population of *Lactobacillus* spp. in the vagina, and this population serves as the first colonization of microbiota during natural birth. After birth, additional microbes are acquired from health-care providers, parents, other relatives, and individuals who come in contact with the baby. This process establishes a microbiome that will continue to evolve over the course of the individual’s life as new microbes colonize and are eliminated from the body. For example, it is estimated that within a 9-hour period, the microbiota of the small intestine can change so that half of the microbial inhabitants will be different.[\[footnote\]](#) The importance of the initial *Lactobacillus* colonization during vaginal child birth is highlighted by studies demonstrating a higher incidence of diseases in individuals born by cesarean section, compared to those born vaginally. Studies have shown that babies born vaginally are predominantly colonized by vaginal *lactobacillus*, whereas babies born by cesarean section are more frequently

colonized by microbes of the normal skin microbiota, including common hospital-acquired pathogens.

C.C. Booijink et al. "High Temporal and Intra-Individual Variation Detected in the Human Ileal Microbiota." *Environmental Microbiology* 12 no. 12 (2010):3213–3227.

Throughout the body, resident microbiotas are important for human health because they occupy niches that might be otherwise taken by pathogenic microorganisms. For instance, *Lactobacillus* spp. are the dominant bacterial species of the normal vaginal microbiota for most women. *Lactobacillus* produce lactic acid, contributing to the acidity of the vagina and inhibiting the growth of pathogenic yeasts. However, when the population of the resident microbiota is decreased for some reason (e.g., because of taking antibiotics), the pH of the vagina increases, making it a more favorable environment for the growth of yeasts such as *Candida albicans*. Antibiotic therapy can also disrupt the microbiota of the intestinal tract and respiratory tract, increasing the risk for secondary infections and/or promoting the long-term carriage and shedding of pathogens.

Note:

- Explain the difference between cooperative and competitive interactions in microbial communities.
- List the types of symbiosis and explain how each population is affected.

Taxonomy and Systematics

Assigning prokaryotes to a certain species is challenging. They do not reproduce sexually, so it is not possible to classify them according to the presence or absence of interbreeding. Also, they do not have many morphological features. Traditionally, the classification of prokaryotes was based on their shape, staining patterns, and biochemical or physiological

differences. More recently, as technology has improved, the nucleotide sequences in genes have become an important criterion of microbial classification.

In 1923, American microbiologist David Hendricks Bergey (1860–1937) published *A Manual in Determinative Bacteriology*. With this manual, he attempted to summarize the information about the kinds of bacteria known at that time, using Latin binomial classification. Bergey also included the morphological, physiological, and biochemical properties of these organisms. His manual has been updated multiple times to include newer bacteria and their properties. It is a great aid in bacterial taxonomy and methods of characterization of bacteria. A more recent sister publication, the five-volume *Bergey's Manual of Systematic Bacteriology*, expands on Bergey's original manual. It includes a large number of additional species, along with up-to-date descriptions of the taxonomy and biological properties of all named prokaryotic taxa. This publication incorporates the approved names of bacteria as determined by the List of Prokaryotic Names with Standing in Nomenclature (LPSN).

Note:



Bergey's Manual of Determinative Bacteriology is now [available](#) online. You can also access a searchable [database](#) of microbial reference strains, published by the American Type Culture Collection (ATCC).

Classification by Staining Patterns

According to their staining patterns, which depend on the properties of their cell walls, bacteria have traditionally been classified into gram-positive, gram-negative, and “atypical,” meaning neither gram-positive nor gram-negative. As explained in [Staining Microscopic Specimens](#), gram-positive bacteria possess a thick peptidoglycan cell wall that retains the primary stain (crystal violet) during the decolorizing step; they remain purple after the gram-stain procedure because the crystal violet dominates the light red/pink color of the secondary counterstain, safranin. In contrast, gram-negative bacteria possess a thin peptidoglycan cell wall that does not prevent the crystal violet from washing away during the decolorizing step; therefore, they appear light red/pink after staining with the safranin. Bacteria that cannot be stained by the standard Gram stain procedure are called atypical bacteria. Included in the atypical category are species of *Mycoplasma* and *Chlamydia*, which lack a cell wall and therefore cannot retain the gram-stain reagents. *Rickettsia* are also considered atypical because they are too small to be evaluated by the Gram stain.

More recently, scientists have begun to further classify gram-negative and gram-positive bacteria. They have added a special group of deeply branching bacteria based on a combination of physiological, biochemical, and genetic features. They also now further classify gram-negative bacteria into Proteobacteria, *Cytophaga-Flavobacterium-Bacteroides* (CFB), and spirochetes.

The deeply branching bacteria are thought to be a very early evolutionary form of bacteria (see [Deeply Branching Bacteria](#)). They live in hot, acidic, ultraviolet-light-exposed, and anaerobic (deprived of oxygen) conditions. Proteobacteria is a phylum of very diverse groups of gram-negative bacteria; it includes some important human pathogens (e.g., *E. coli* and *Bordetella pertussis*). The CFB group of bacteria includes components of the normal human gut microbiota, like *Bacteroides*. The spirochetes are spiral-shaped bacteria and include the pathogen *Treponema pallidum*, which causes syphilis. We will characterize these groups of bacteria in more detail later in the chapter.

Based on their prevalence of guanine and cytosine nucleotides, gram-positive bacteria are also classified into low G+C and high G+C gram-

positive bacteria. The low G+C gram-positive bacteria have less than 50% of guanine and cytosine nucleotides in their DNA. They include human pathogens, such as those that cause anthrax (*Bacillus anthracis*), tetanus (*Clostridium tetani*), and listeriosis (*Listeria monocytogenes*). High G+C gram-positive bacteria, which have more than 50% guanine and cytosine nucleotides in their DNA, include the bacteria that cause diphtheria (*Corynebacterium diphtheriae*), tuberculosis (*Mycobacterium tuberculosis*), and other diseases.

The classifications of prokaryotes are constantly changing as new species are being discovered. We will describe them in more detail, along with the diseases they cause, in later sections and chapters.

Note:

- How do scientists classify prokaryotes?

Note:

Human Microbiome Project

The Human Microbiome Project was launched by the National Institutes of Health (NIH) in 2008. One main goal of the project is to create a large repository of the gene sequences of important microbes found in humans, helping biologists and clinicians understand the dynamics of the human microbiome and the relationship between the human microbiota and diseases. A network of labs working together has been compiling the data from swabs of several areas of the skin, gut, and mouth from hundreds of individuals.

One of the challenges in understanding the human microbiome has been the difficulty of culturing many of the microbes that inhabit the human body. It has been estimated that we are only able to culture 1% of the bacteria in nature and that we are unable to grow the remaining 99%. To address this challenge, researchers have used metagenomic analysis, which studies genetic material harvested directly from microbial communities, as

opposed to that of individual species grown in a culture. This allows researchers to study the genetic material of all microbes in the microbiome, rather than just those that can be cultured.[\[footnote\]](#)

National Institutes of Health. “Human Microbiome Project. Overview.”

<http://commonfund.nih.gov/hmp/overview>. Accessed June 7, 2016.

One important achievement of the Human Microbiome Project is establishing the first reference database on microorganisms living in and on the human body. Many of the microbes in the microbiome are beneficial, but some are not. It was found, somewhat unexpectedly, that all of us have some serious microbial pathogens in our microbiota. For example, the conjunctiva of the human eye contains 24 genera of bacteria and numerous pathogenic species.[\[footnote\]](#) A healthy human mouth contains a number of species of the genus *Streptococcus*, including pathogenic species *S. pyogenes* and *S. pneumoniae*.[\[footnote\]](#) This raises the question of why certain prokaryotic organisms exist commensally in certain individuals but act as deadly pathogens in others. Also unexpected was the number of organisms that had never been cultured. For example, in one metagenomic study of the human gut microbiota, 174 new species of bacteria were identified.[\[footnote\]](#)

Q. Dong et al. “Diversity of Bacteria at Healthy Human Conjunctiva.”

Investigative Ophthalmology & Visual Science 52 no. 8 (2011):5408–5413.

F.E. Dewhirst et al. “The Human Oral Microbiome.” *Journal of Bacteriology* 192 no. 19 (2010):5002–5017.

J.C. Lagier et al. “Microbial Culturomics: Paradigm Shift in the Human Gut Microbiome Study.” *Clinical Microbiology and Infection* 18 no. 12 (2012):1185–1193.

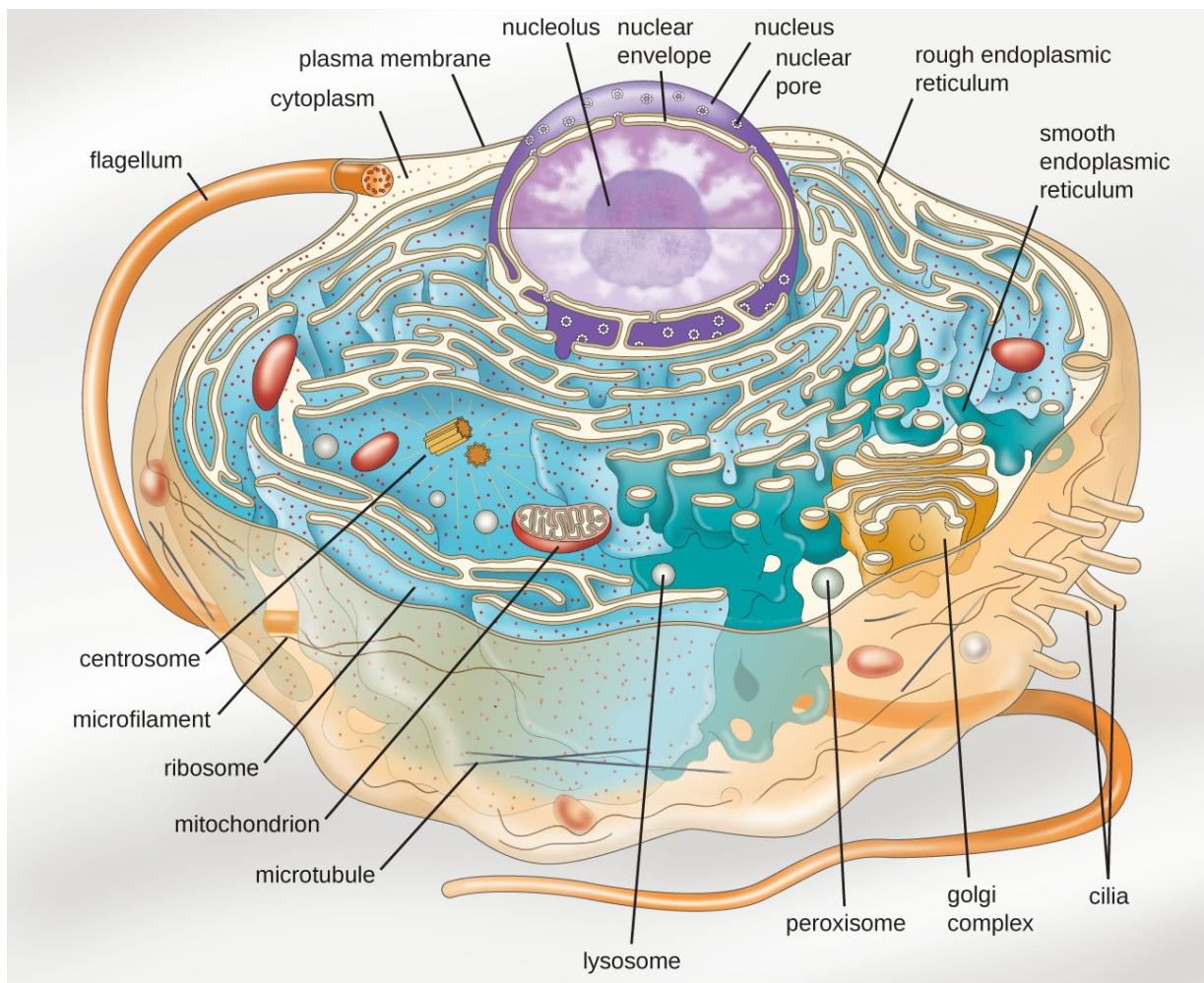
Another goal for the near future is to characterize the human microbiota in patients with different diseases and to find out whether there are any relationships between the contents of an individual’s microbiota and risk for or susceptibility to specific diseases. Analyzing the microbiome in a person with a specific disease may reveal new ways to fight diseases.

Unique Characteristics of Eukaryotic Cells

LEARNING OBJECTIVES

- Explain the distinguishing characteristics of eukaryotic cells
- Describe internal and external structures of prokaryotic cells in terms of their physical structure, chemical structure, and function
- Identify and describe structures and organelles unique to eukaryotic cells
- Compare and contrast similar structures found in prokaryotic and eukaryotic cells

Eukaryotic organisms include protozoans, algae, fungi, plants, and animals. Some eukaryotic cells are independent, single-celled microorganisms, whereas others are part of multicellular organisms. The cells of eukaryotic organisms have several distinguishing characteristics. Above all, eukaryotic cells are defined by the presence of a nucleus surrounded by a complex nuclear membrane. Also, eukaryotic cells are characterized by the presence of membrane-bound organelles in the cytoplasm. Organelles such as mitochondria, the endoplasmic reticulum (ER), Golgi apparatus, lysosomes, and peroxisomes are held in place by the **cytoskeleton**, an internal network that supports transport of intracellular components and helps maintain cell shape ([\[link\]](#)). The genome of eukaryotic cells is packaged in multiple, rod-shaped chromosomes as opposed to the single, circular-shaped chromosome that characterizes most prokaryotic cells. [\[link\]](#) compares the characteristics of eukaryotic cell structures with those of bacteria and archaea.



An illustration of a generalized, single-celled eukaryotic organism. Note that cells of eukaryotic organisms vary greatly in terms of structure and function, and a particular cell may not have all of the structures shown here.

Summary of Cell Structures

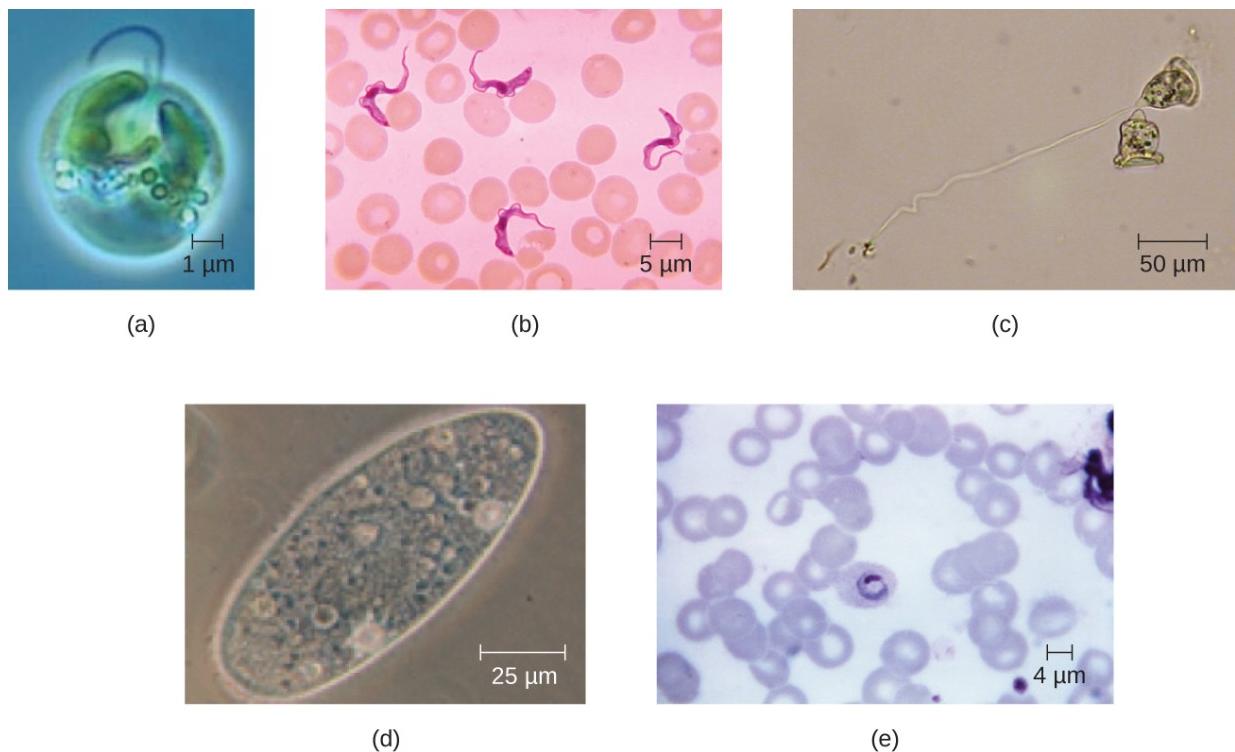
Cell Structure	Prokaryotes		Eukaryotes
	Bacteria	Archaea	
Size	~0.5–1 μM	~0.5–1 μM	~5–20 μM

Summary of Cell Structures			
Cell Structure	Prokaryotes		Eukaryotes
	Bacteria	Archaea	
Surface area-to-volume ratio	High	High	Low
Nucleus	No	No	Yes
Genome characteristics	<ul style="list-style-type: none"> Single chromosome Circular Haploid Lacks histones 	<ul style="list-style-type: none"> Single chromosome Circular Haploid Contains histones 	<ul style="list-style-type: none"> Multiple chromosomes Linear Haploid or diploid Contains histones
Cell division	Binary fission	Binary fission	Mitosis, meiosis
Membrane lipid composition	<ul style="list-style-type: none"> Ester-linked Straight-chain fatty acids Bilayer 	<ul style="list-style-type: none"> Ether-linked Branched isoprenoids Bilayer or monolayer 	<ul style="list-style-type: none"> Ester-linked Straight-chain fatty acids Sterols Bilayer
Cell wall composition	<ul style="list-style-type: none"> Peptidoglycan, or None 	<ul style="list-style-type: none"> Pseudopeptidoglycan, or Glycopeptide, or Polysaccharide, or Protein (S-layer), or None 	<ul style="list-style-type: none"> Cellulose (plants, some algae) Chitin (molluscs, insects, crustaceans, and fungi) Silica (some algae) Most others lack cell walls

Summary of Cell Structures			
Cell Structure	Prokaryotes		Eukaryotes
	Bacteria	Archaea	
Motility structures	Rigid spiral flagella composed of flagellin	Rigid spiral flagella composed of archaeal flagellins	Flexible flagella and cilia composed of microtubules
Membrane-bound organelles	No	No	Yes
Endomembrane system	No	No	Yes (ER, Golgi, lysosomes)
Ribosomes	70S	70S	<ul style="list-style-type: none"> • 80S in cytoplasm and rough ER • 70S in mitochondria, chloroplasts

Cell Morphologies

Eukaryotic cells display a wide variety of different cell morphologies. Possible shapes include spheroid, ovoid, cuboidal, cylindrical, flat, lenticular, fusiform, discoidal, crescent, ring stellate, and polygonal ([\[link\]](#)). Some eukaryotic cells are irregular in shape, and some are capable of changing shape. The shape of a particular type of eukaryotic cell may be influenced by factors such as its primary function, the organization of its cytoskeleton, the viscosity of its cytoplasm, the rigidity of its cell membrane or cell wall (if it has one), and the physical pressure exerted on it by the surrounding environment and/or adjoining cells.



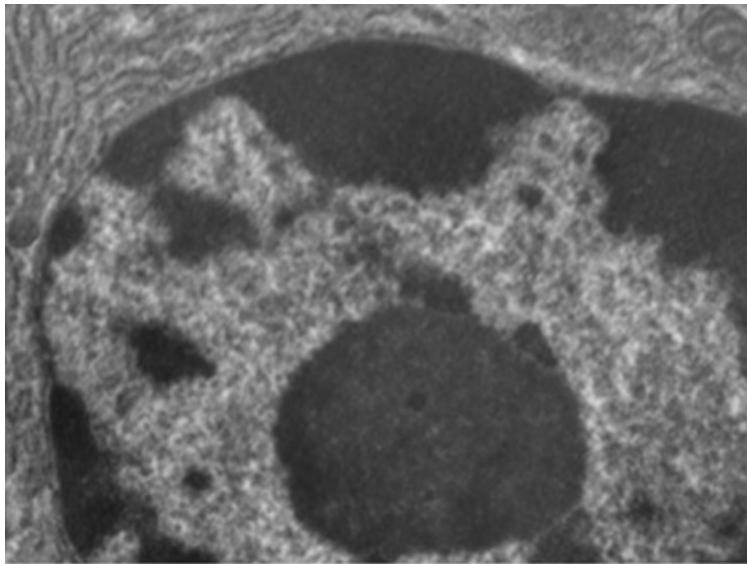
Eukaryotic cells come in a variety of cell shapes. (a) Spheroid *Chromulina* alga. (b) Fusiform shaped *Trypanosoma*. (c) Bell-shaped *Vorticella*. (d) Ovoid *Paramecium*. (e) Ring-shaped *Plasmodium ovale*. (credit a: modification of work by NOAA; credit b, e: modification of work by Centers for Disease Control and Prevention)

Note:

- Identify two differences between eukaryotic and prokaryotic cells.

Nucleus

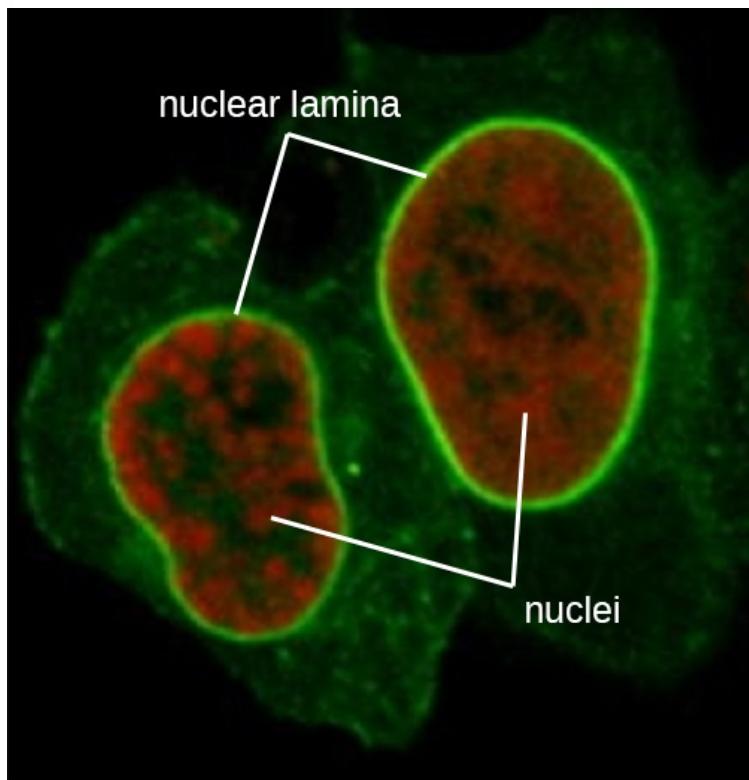
Unlike prokaryotic cells, in which DNA is loosely contained in the nucleoid region, eukaryotic cells possess a **nucleus**, which is surrounded by a complex nuclear membrane that houses the DNA genome ([\[link\]](#)). By containing the cell's DNA, the nucleus ultimately controls all activities of the cell and also serves an essential role in reproduction and heredity. Eukaryotic cells typically have their DNA organized into multiple linear chromosomes. The DNA within the nucleus is highly organized and condensed to fit inside the nucleus, which is accomplished by wrapping the DNA around proteins called histones.



Eukaryotic cells have a well-defined nucleus. The nucleus of this mammalian lung cell is the large, dark, oval-shaped structure in the lower half of the image.

Although most eukaryotic cells have only one nucleus, exceptions exist. For example, protozoans of the genus *Paramecium* typically have two complete nuclei: a small nucleus that is used for reproduction (micronucleus) and a large nucleus that directs cellular metabolism (macronucleus). Additionally, some fungi transiently form cells with two nuclei, called heterokaryotic cells, during sexual reproduction. Cells whose nuclei divide, but whose cytoplasm does not, are called **coenocytes**.

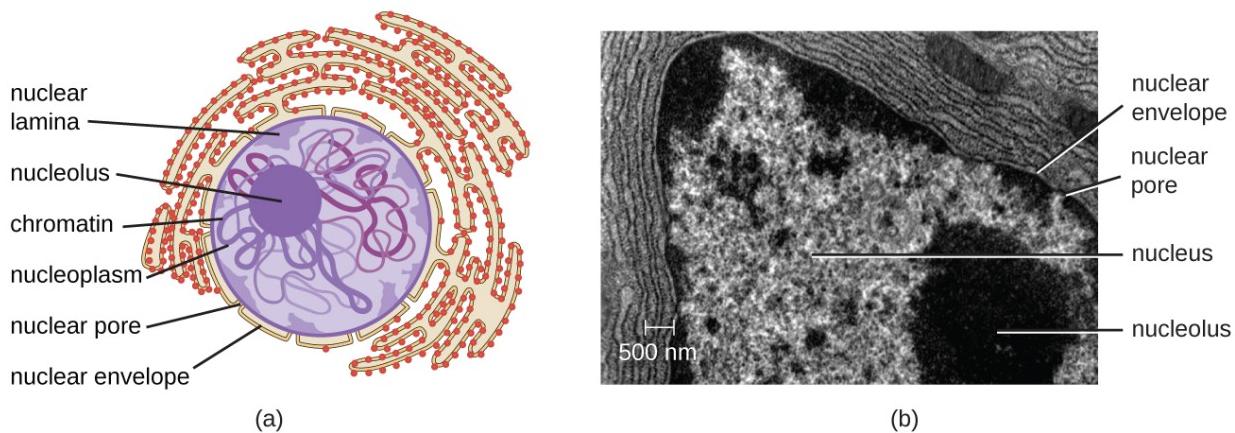
The nucleus is bound by a complex **nuclear membrane**, often called the **nuclear envelope**, that consists of two distinct lipid bilayers that are contiguous with each other ([\[link\]](#)). Despite these connections between the inner and outer membranes, each membrane contains unique lipids and proteins on its inner and outer surfaces. The nuclear envelope contains nuclear pores, which are large, rosette-shaped protein complexes that control the movement of materials into and out of the nucleus. The overall shape of the nucleus is determined by the **nuclear lamina**, a meshwork of intermediate filaments found just inside the nuclear envelope membranes. Outside the nucleus, additional intermediate filaments form a looser mesh and serve to anchor the nucleus in position within the cell.



In this fluorescent microscope image, all the intermediate filaments have been stained with a bright green fluorescent stain. The nuclear lamina is the intense bright green ring around the faint red nuclei.

Nucleolus

The **nucleolus** is a dense region within the nucleus where ribosomal RNA (rRNA) biosynthesis occurs. In addition, the nucleolus is also the site where assembly of ribosomes begins. Preribosomal complexes are assembled from rRNA and proteins in the nucleolus; they are then transported out to the cytoplasm, where ribosome assembly is completed ([\[link\]](#)).



(a) The nucleolus is the dark, dense area within the nucleus. It is the site of rRNA synthesis and preribosomal assembly. (b) Electron micrograph showing the nucleolus.

Ribosomes

Ribosomes found in eukaryotic organelles such as mitochondria or chloroplasts have 70S ribosomes—the same size as prokaryotic ribosomes. However, nonorganelle-associated ribosomes in eukaryotic cells are **80S ribosomes**, composed of a 40S small subunit and a 60S large subunit. In terms of size and composition, this makes them distinct from the ribosomes of prokaryotic cells.

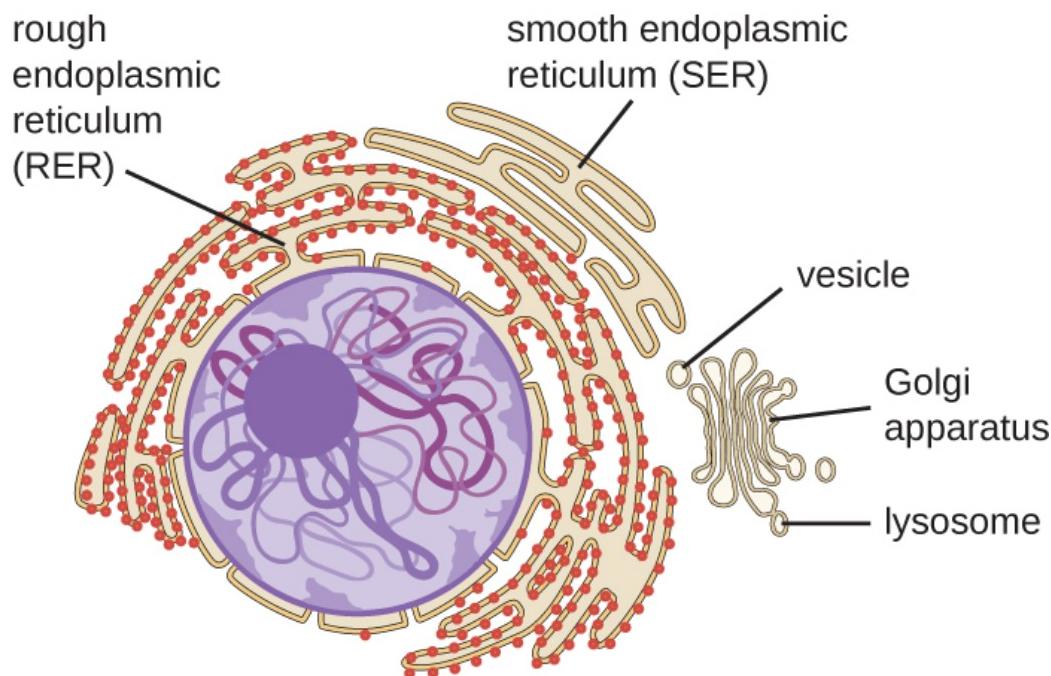
The two types of nonorganelle-associated eukaryotic ribosomes are defined by their location in the cell: **free ribosomes** and **membrane-bound ribosomes**. Free ribosomes are found in the cytoplasm and serve to synthesize water-soluble proteins; membrane-bound ribosomes are found attached to the rough endoplasmic reticulum and make proteins for insertion into the cell membrane or proteins destined for export from the cell.

The differences between eukaryotic and prokaryotic ribosomes are clinically relevant because certain antibiotic drugs are designed to target one or the other. For example, cycloheximide targets eukaryotic action, whereas chloramphenicol targets prokaryotic ribosomes.[\[footnote\]](#) Since human cells are eukaryotic, they generally are not harmed by antibiotics that destroy the prokaryotic ribosomes in bacteria. However, sometimes negative side effects may occur because mitochondria in human cells contain prokaryotic ribosomes.

A.E. Barnhill, M.T. Brewer, S.A. Carlson. “Adverse Effects of Antimicrobials via Predictable or Idiosyncratic Inhibition of Host Mitochondrial Components.” *Antimicrobial Agents and Chemotherapy* 56 no. 8 (2012):4046–4051.

Endomembrane System

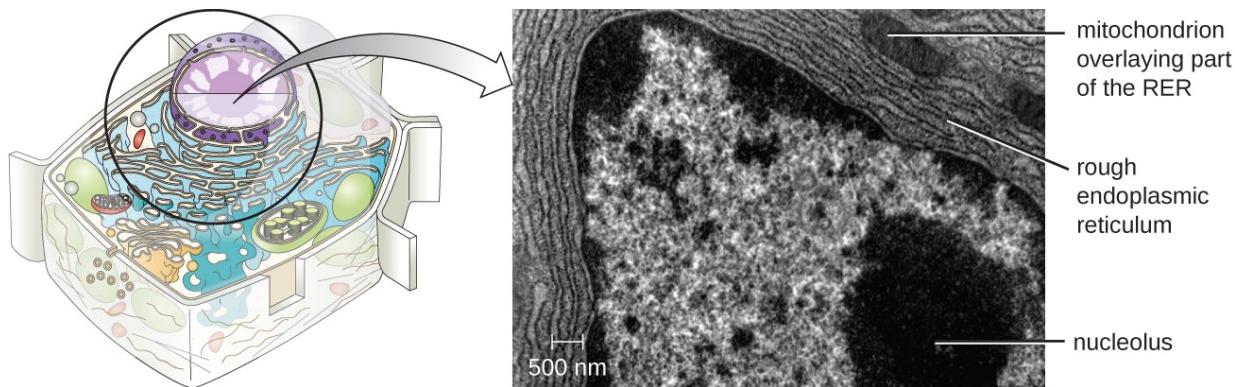
The **endomembrane system**, unique to eukaryotic cells, is a series of membranous tubules, sacs, and flattened disks that synthesize many cell components and move materials around within the cell ([\[link\]](#)). Because of their larger cell size, eukaryotic cells require this system to transport materials that cannot be dispersed by diffusion alone. The endomembrane system comprises several organelles and connections between them, including the endoplasmic reticulum, Golgi apparatus, lysosomes, and vesicles.



The endomembrane system is composed of a series of membranous intracellular structures that facilitate movement of materials throughout the cell and to the cell membrane.

Endoplasmic Reticulum

The **endoplasmic reticulum (ER)** is an interconnected array of tubules and **cisternae** (flattened sacs) with a single lipid bilayer ([\[link\]](#)). The spaces inside of the cisternae are called **lumen** of the ER. There are two types of ER, **rough endoplasmic reticulum (RER)** and **smooth endoplasmic reticulum (SER)**. These two different types of ER are sites for the synthesis of distinctly different types of molecules. RER is studded with ribosomes bound on the cytoplasmic side of the membrane. These ribosomes make proteins destined for the plasma membrane ([\[link\]](#)). Following synthesis, these proteins are inserted into the membrane of the RER. Small sacs of the RER containing these newly synthesized proteins then bud off as **transport vesicles** and move either to the Golgi apparatus for further processing, directly to the plasma membrane, to the membrane of another organelle, or out of the cell. Transport vesicles are single-lipid, bilayer, membranous spheres with hollow interiors that carry molecules. SER does not have ribosomes and, therefore, appears “smooth.” It is involved in biosynthesis of lipids, carbohydrate metabolism, and detoxification of toxic compounds within the cell.



The rough endoplasmic reticulum is studded with ribosomes for the synthesis of membrane proteins (which give it its rough appearance).

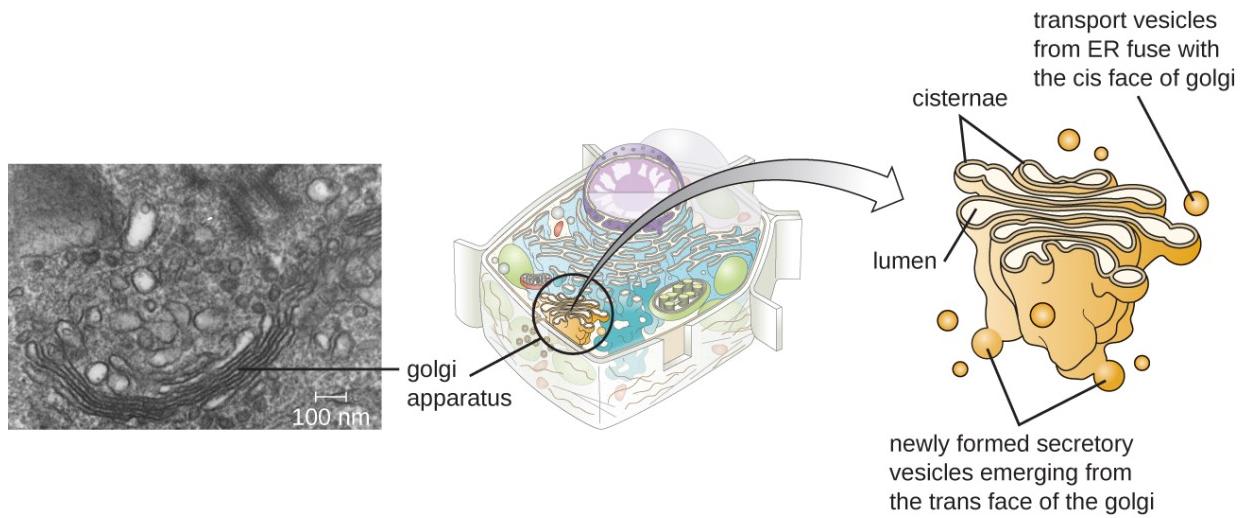
Golgi Apparatus

The Golgi apparatus was discovered within the endomembrane system in 1898 by Italian scientist Camillo Golgi (1843–1926), who developed a novel staining technique that showed stacked membrane structures within the cells of *Plasmodium*, the causative agent of malaria. The **Golgi apparatus** is composed of a series of membranous disks called dictyosomes, each having a single lipid bilayer, that are stacked together ([\[link\]](#)).

Enzymes in the Golgi apparatus modify lipids and proteins transported from the ER to the Golgi, often adding carbohydrate components to them, producing glycolipids, glycoproteins, or proteoglycans. Glycolipids and glycoproteins are often inserted into the plasma membrane and are important for signal recognition by other cells or infectious particles. Different types of cells can be distinguished from one another by the structure and arrangement of the glycolipids and glycoproteins contained in their plasma membranes. These glycolipids and glycoproteins commonly also serve as cell surface receptors.

Transport vesicles leaving the ER fuse with a Golgi apparatus on its receiving, or *cis*, face. The proteins are processed within the Golgi apparatus, and then additional transport vesicles containing the modified proteins and lipids pinch off from the Golgi apparatus on its outgoing, or *trans*, face. These outgoing vesicles move to and fuse with the plasma membrane or the membrane of other organelles.

Exocytosis is the process by which **secretory vesicles** (spherical membranous sacs) release their contents to the cell's exterior ([\[link\]](#)). All cells have constitutive secretory pathways in which secretory vesicles transport soluble proteins that are released from the cell continually (constitutively). Certain specialized cells also have regulated secretory pathways, which are used to store soluble proteins in secretory vesicles. Regulated secretion involves substances that are only released in response to certain events or signals. For example, certain cells of the human immune system (e.g., mast cells) secrete histamine in response to the presence of foreign objects or pathogens in the body. Histamine is a compound that triggers various mechanisms used by the immune system to eliminate pathogens.



A transmission electron micrograph (left) of a Golgi apparatus in a white blood cell. The illustration (right) shows the cup-shaped, stacked disks and several transport vesicles. The Golgi apparatus modifies lipids and proteins, producing glycolipids and glycoproteins, respectively, which are commonly inserted into the plasma membrane.

Lysosomes

In the 1960s, Belgian scientist Christian de Duve (1917–2013) discovered **lysosomes**, membrane-bound organelles of the endomembrane system that contain digestive enzymes. Certain types of eukaryotic cells use lysosomes to break down various particles, such as food, damaged organelles or cellular debris, microorganisms, or immune complexes. Compartmentalization of the digestive enzymes within the lysosome allows the cell to efficiently digest matter without harming the cytoplasmic components of the cell.

Note:

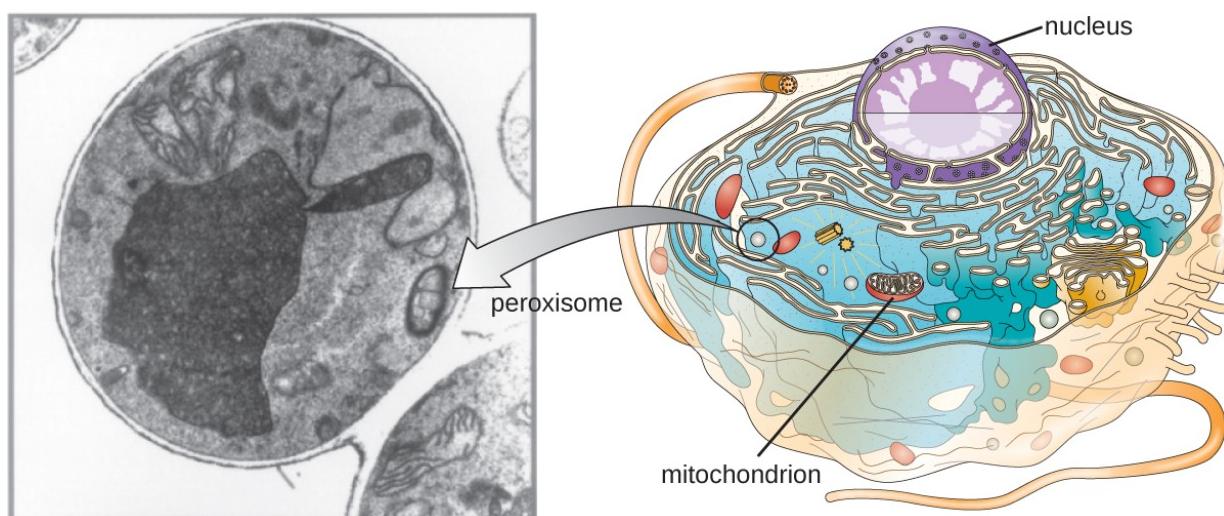
- Name the components of the endomembrane system and describe the function of each component.

Peroxisomes

Christian de Duve is also credited with the discovery of **peroxisomes**, membrane-bound organelles that are not part of the endomembrane system ([\[link\]](#)). Peroxisomes form independently in the cytoplasm from the synthesis of peroxin proteins by free ribosomes and the incorporation of these peroxin proteins into existing peroxisomes. Growing peroxisomes then divide by a process similar to binary fission.

Peroxisomes were first named for their ability to produce hydrogen peroxide, a highly reactive molecule that helps to break down molecules such as uric acid, amino acids, and fatty acids. Peroxisomes also possess the enzyme catalase, which can degrade hydrogen peroxide. Along with the SER, peroxisomes also play a role in lipid biosynthesis. Like lysosomes, the compartmentalization of these degradative molecules within an organelle helps protect the cytoplasmic contents from unwanted damage.

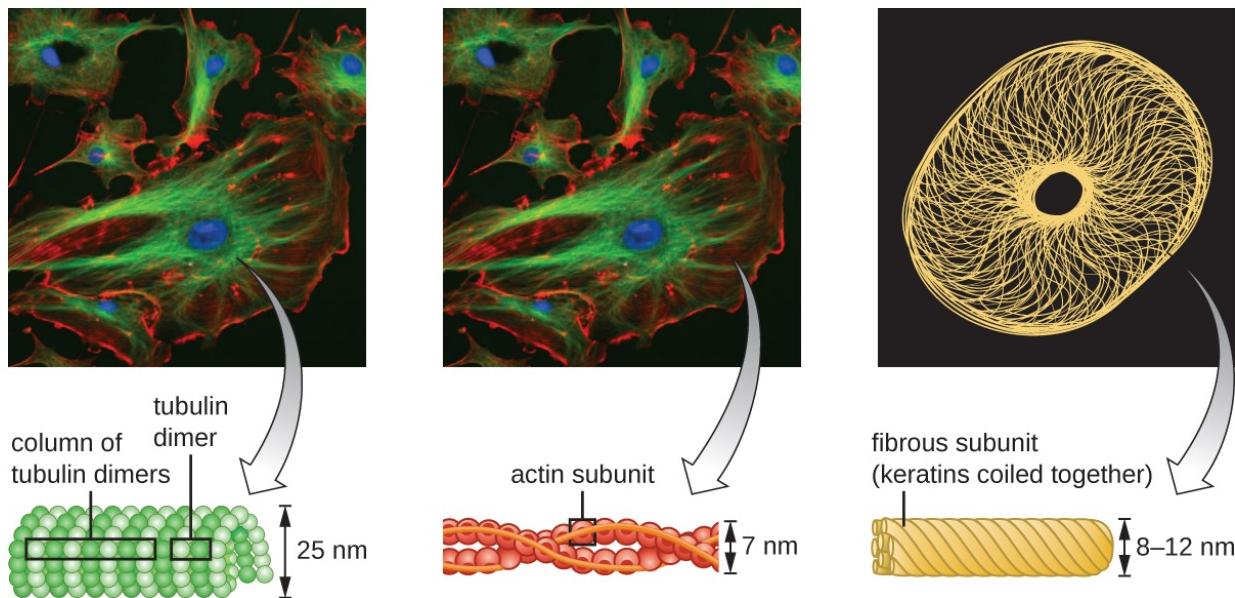
The peroxisomes of certain organisms are specialized to meet their particular functional needs. For example, glyoxysomes are modified peroxisomes of yeasts and plant cells that perform several metabolic functions, including the production of sugar molecules. Similarly, glycosomes are modified peroxisomes made by certain trypanosomes, the pathogenic protozoans that cause Chagas disease and African sleeping sickness.



A transmission electron micrograph (left) of a cell containing a peroxisome. The illustration (right) shows the location of peroxisomes in a cell. These eukaryotic structures play a role in lipid biosynthesis and breaking down various molecules. They may also have other specialized functions depending on the cell type. (credit “micrograph”: modification of work by American Society for Microbiology)

Cytoskeleton

Eukaryotic cells have an internal cytoskeleton made of **microfilaments**, **intermediate filaments**, and **microtubules**. This matrix of fibers and tubes provides structural support as well as a network over which materials can be transported within the cell and on which organelles can be anchored ([\[link\]](#)). For example, the process of exocytosis involves the movement of a vesicle via the cytoskeletal network to the plasma membrane, where it can release its contents.

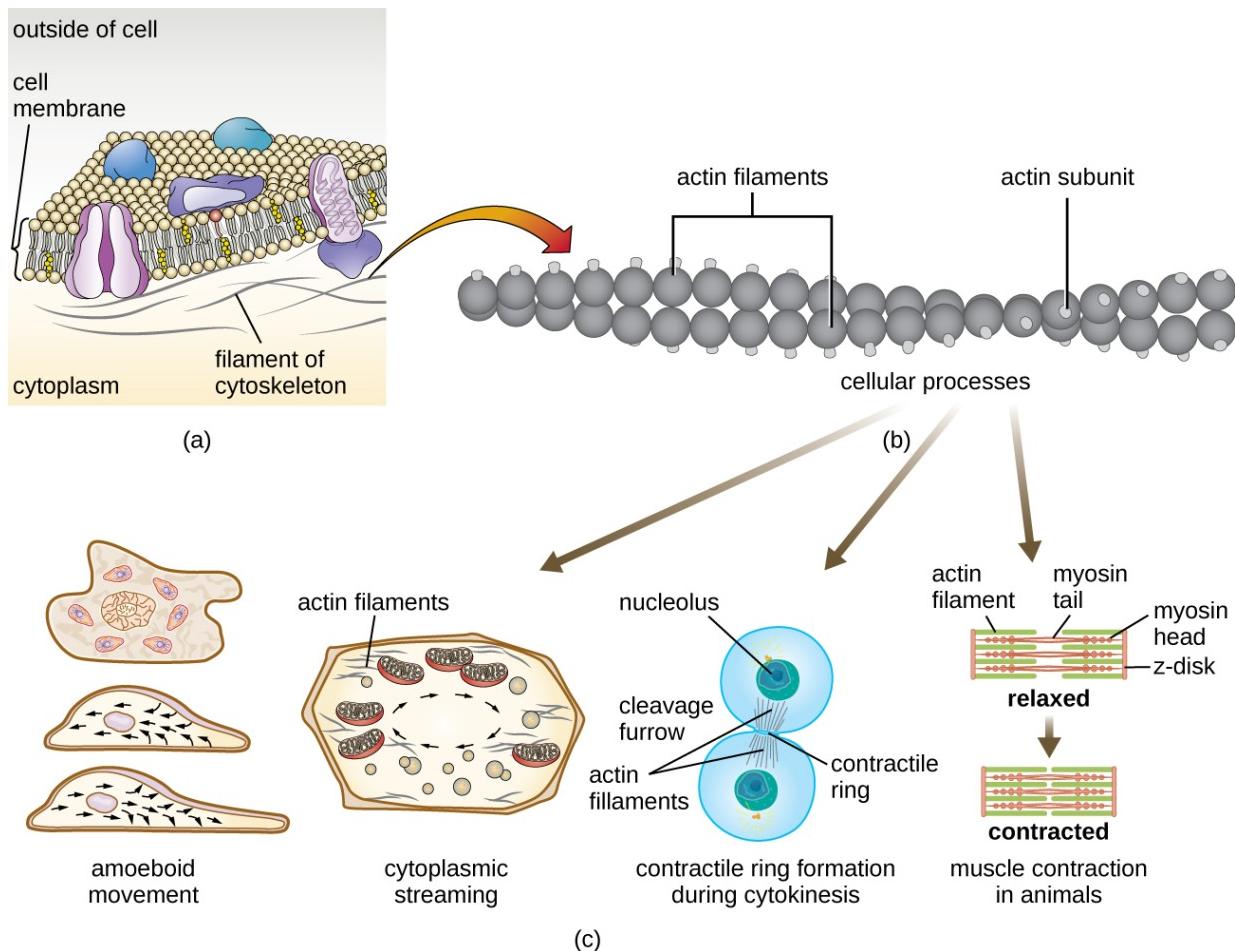


The cytoskeleton is a network of microfilaments, intermediate filaments, and microtubules found throughout the cytoplasm of a eukaryotic cell. In these fluorescently labeled animal cells, the microtubules are green, the actin microfilaments are red, the nucleus is blue, and keratin (a type of intermediate filament) is yellow.

Microfilaments are composed of two intertwined strands of actin, each composed of **actin** monomers forming filamentous cables 6 nm in diameter [footnote] ([link]). The actin filaments work together with motor proteins, like myosin, to effect muscle contraction in animals or the amoeboid movement of some eukaryotic microbes. In ameoboid organisms, actin can be found in two forms: a stiffer, polymerized, gel form and a more fluid, unpolymerized soluble form. Actin in the gel form creates stability in the ectoplasm, the gel-like area of cytoplasm just inside the plasma membrane of ameoboid protozoans.

Fuchs E, Cleveland DW. "A Structural Scaffolding of Intermediate Filaments in Health and Disease." *Science* 279 no. 5350 (1998):514–519.

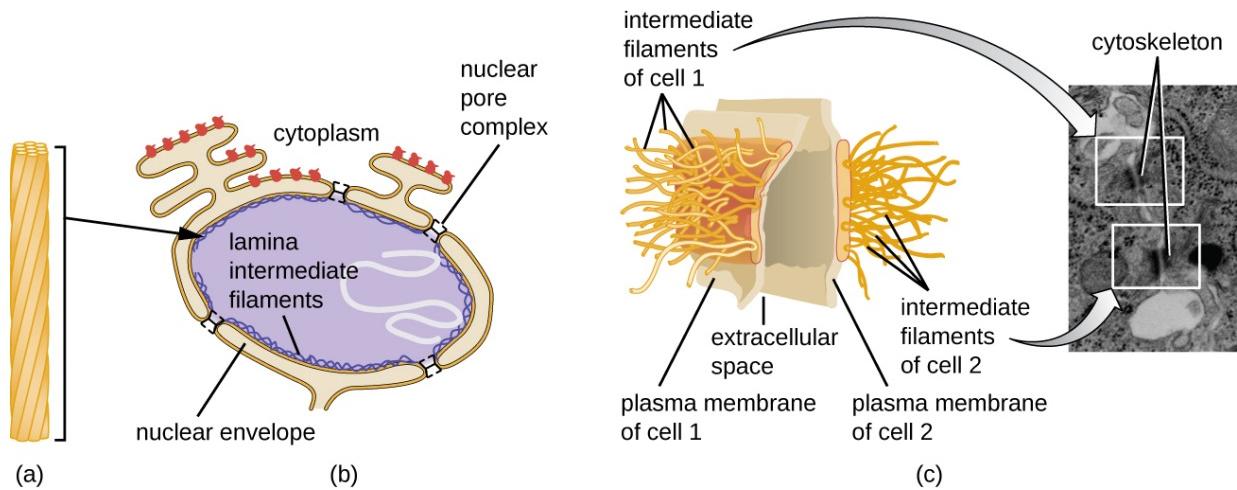
Temporary extensions of the cytoplasmic membrane called **pseudopodia** (meaning “false feet”) are produced through the forward flow of soluble actin filaments into the pseudopodia, followed by the gel-sol cycling of the actin filaments, resulting in cell motility. Once the cytoplasm extends outward, forming a pseudopodium, the remaining cytoplasm flows up to join the leading edge, thereby creating forward locomotion. Beyond amoeboid movement, microfilaments are also involved in a variety of other processes in eukaryotic cells, including cytoplasmic streaming (the movement or circulation of cytoplasm within the cell), cleavage furrow formation during cell division, and muscle movement in animals ([link]). These functions are the result of the dynamic nature of microfilaments, which can polymerize and depolymerize relatively easily in response to cellular signals, and their interactions with molecular motors in different types of eukaryotic cells.



(a) A microfilament is composed of a pair of actin filaments. (b) Each actin filament is a string of polymerized actin monomers. (c) The dynamic nature of actin, due to its polymerization and depolymerization and its association with myosin, allows microfilaments to be involved in a variety of cellular processes, including amoeboid movement, cytoplasmic streaming, contractile ring formation during cell division, and muscle contraction in animals.

Intermediate filaments ([\[link\]](#)) are a diverse group of cytoskeletal filaments that act as cables within the cell. They are termed “intermediate” because their 10-nm diameter is thicker than that of actin but thinner than that of microtubules. [\[footnote\]](#) They are composed of several strands of polymerized subunits that, in turn, are made up of a wide variety of monomers. Intermediate filaments tend to be more permanent in the cell and maintain the position of the nucleus. They also form the nuclear lamina (lining or layer) just inside the nuclear envelope. Additionally, intermediate filaments play a role in anchoring cells together in animal tissues. The intermediate filament protein desmin is found in desmosomes, the protein structures that join muscle cells together and help them resist external physical forces. The intermediate filament protein keratin is a structural protein found in hair, skin, and nails.

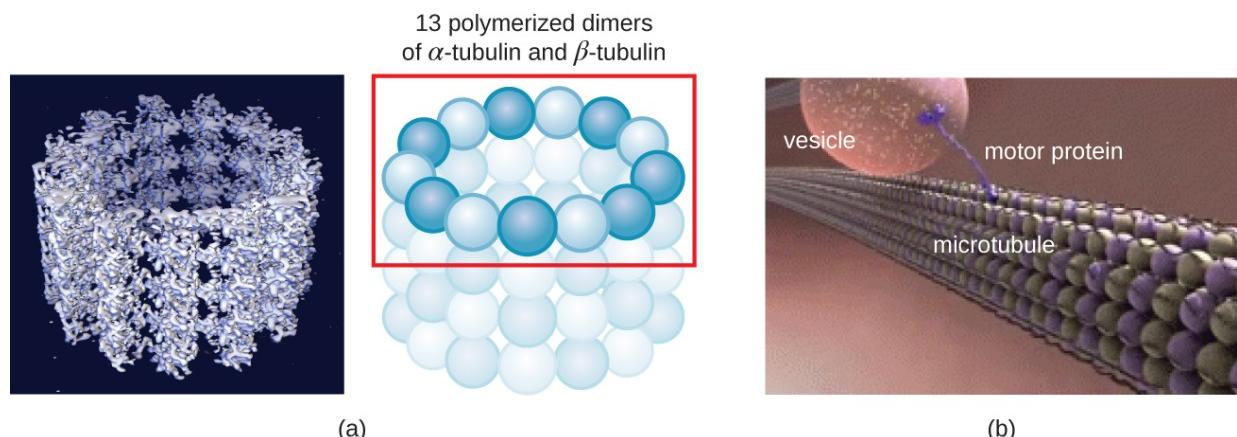
E. Fuchs, D.W. Cleveland. “A Structural Scaffolding of Intermediate Filaments in Health and Disease.” *Science* 279 no. 5350 (1998):514–519.



(a) Intermediate filaments are composed of multiple strands of polymerized subunits. They are more permanent than other cytoskeletal structures and serve a variety of functions. (b) Intermediate filaments form much of the nuclear lamina. (c) Intermediate filaments form the desmosomes between cells in some animal tissues. (credit c “illustration”: modification of work by Mariana Ruiz Villareal)

Microtubules ([\[link\]](#)) are a third type of cytoskeletal fiber composed of tubulin dimers (α tubulin and β tubulin). These form hollow tubes 23 nm in diameter that are used as girders within the cytoskeleton. [\[footnote\]](#) Like microfilaments, microtubules are dynamic and have the ability to rapidly assemble and disassemble. Microtubules also work with motor proteins (such as dynein and kinesin) to move organelles and vesicles around within the cytoplasm. Additionally, microtubules are the main components of eukaryotic flagella and cilia, composing both the filament and the basal body components ([\[link\]](#)).

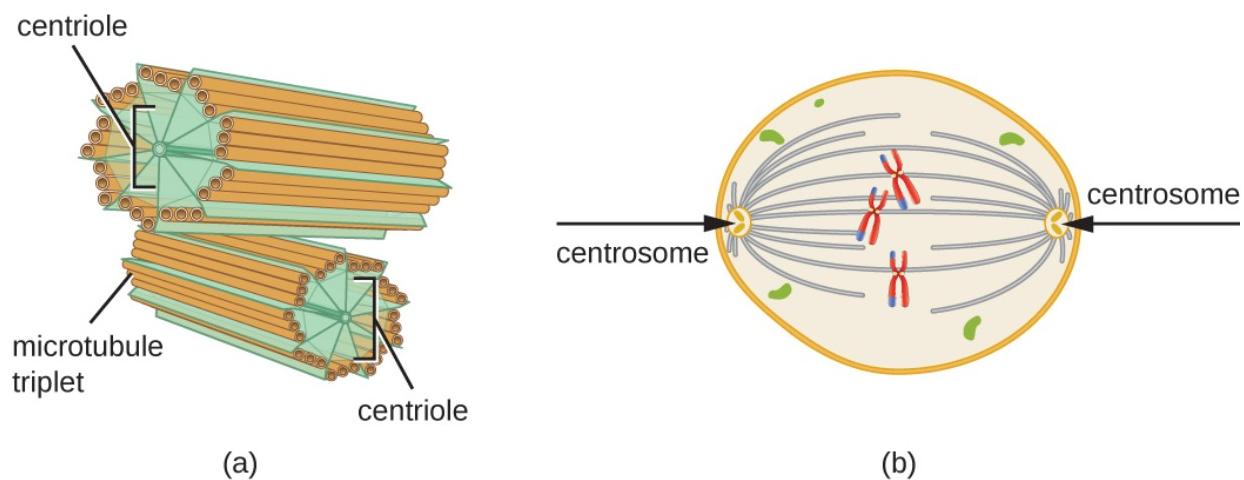
E. Fuchs, D.W. Cleveland. “A Structural Scaffolding of Intermediate Filaments in Health and Disease.” *Science* 279 no. 5350 (1998):514–519.



(a) Microtubules are hollow structures composed of polymerized tubulin dimers. (b) They are involved in several cellular processes, including the movement of organelles throughout the

cytoplasm. Motor proteins carry organelles along microtubule tracks that crisscross the entire cell. (credit b: modification of work by National Institute on Aging)

In addition, microtubules are involved in cell division, forming the mitotic spindle that serves to separate chromosomes during mitosis and meiosis. The mitotic spindle is produced by two **centrosomes**, which are essentially microtubule-organizing centers, at opposite ends of the cell. Each centrosome is composed of a pair of **centrioles** positioned at right angles to each other, and each centriole is an array of nine parallel microtubules arranged in triplets ([\[link\]](#)).



(a) A centrosome is composed of two centrioles positioned at right angles to each other. Each centriole is composed of nine triplets of microtubules held together by accessory proteins. (b) In animal cells, the centrosomes (arrows) serve as microtubule-organizing centers of the mitotic spindle during mitosis.

Note:

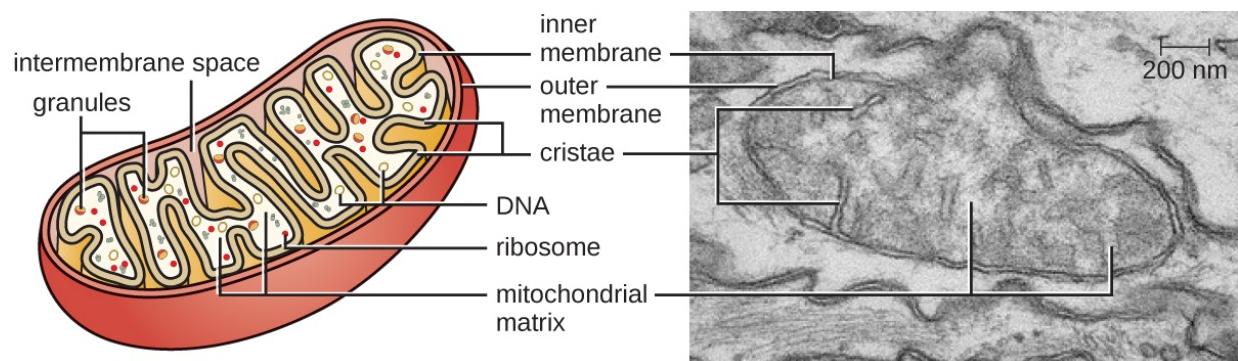
- Compare and contrast the three types of cytoskeletal structures described in this section.

Mitochondria

The large, complex organelles in which aerobic cellular respiration occurs in eukaryotic cells are called **mitochondria** ([\[link\]](#)). The term “mitochondrion” was first coined by German microbiologist Carl Benda in 1898 and was later connected with the process of respiration by Otto Warburg in 1913. Scientists during the 1960s discovered that mitochondria have their own genome and 70S ribosomes. The mitochondrial genome was found to be bacterial, when it was sequenced in 1976. These findings

ultimately supported the endosymbiotic theory proposed by Lynn Margulis, which states that mitochondria originally arose through an endosymbiotic event in which a bacterium capable of aerobic cellular respiration was taken up by phagocytosis into a host cell and remained as a viable intracellular component.

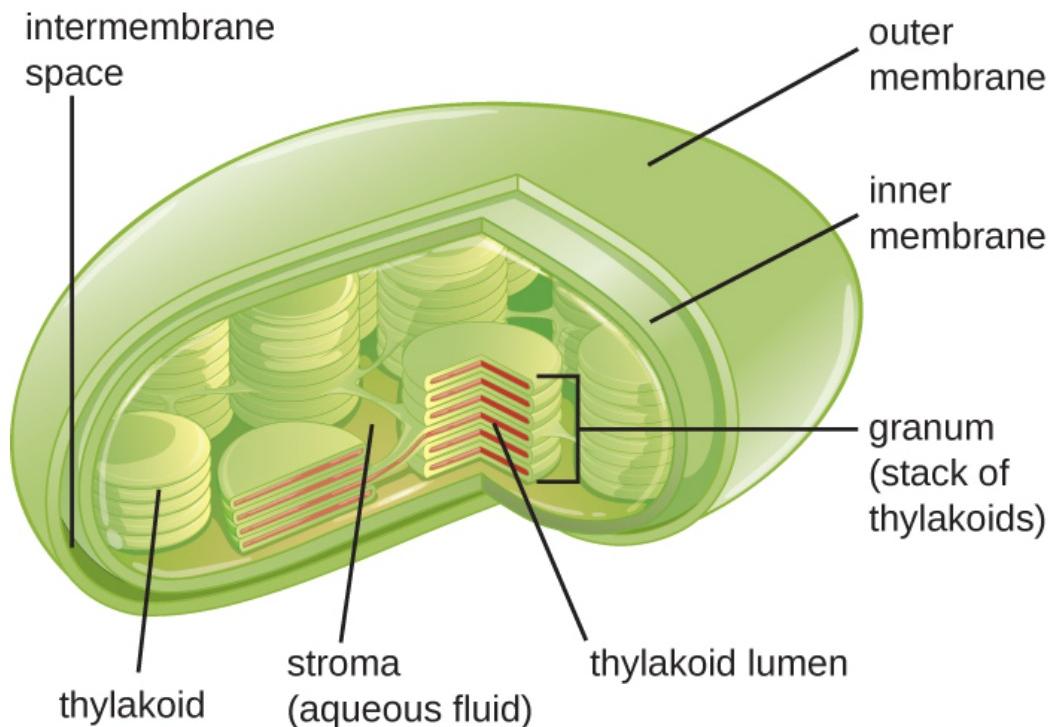
Each mitochondrion has two lipid membranes. The outer membrane is a remnant of the original host cell's membrane structures. The inner membrane was derived from the bacterial plasma membrane. The electron transport chain for aerobic respiration uses integral proteins embedded in the inner membrane. The **mitochondrial matrix**, corresponding to the location of the original bacterium's cytoplasm, is the current location of many metabolic enzymes. It also contains mitochondrial DNA and 70S ribosomes. Invaginations of the inner membrane, called cristae, evolved to increase surface area for the location of biochemical reactions. The folding patterns of the cristae differ among various types of eukaryotic cells and are used to distinguish different eukaryotic organisms from each other.



Each mitochondrion is surrounded by two membranes, the inner of which is extensively folded into cristae and is the site of the intermembrane space. The mitochondrial matrix contains the mitochondrial DNA, ribosomes, and metabolic enzymes. The transmission electron micrograph of a mitochondrion, on the right, shows both membranes, including cristae and the mitochondrial matrix. (credit "micrograph": modification of work by Matthew Britton; scale-bar data from Matt Russell)

Chloroplasts

Plant cells and algal cells contain **chloroplasts**, the organelles in which photosynthesis occurs ([\[link\]](#)). All chloroplasts have at least three membrane systems: the outer membrane, the inner membrane, and the thylakoid membrane system. Inside the outer and inner membranes is the chloroplast **stroma**, a gel-like fluid that makes up much of a chloroplast's volume, and in which the **thylakoid** system floats. The thylakoid system is a highly dynamic collection of folded membrane sacs. It is where the green photosynthetic pigment chlorophyll is found and the light reactions of photosynthesis occur. In most plant chloroplasts, the thylakoids are arranged in stacks called grana (singular: granum), whereas in some algal chloroplasts, the thylakoids are free floating.



Photosynthesis takes place in chloroplasts, which have an outer membrane and an inner membrane. Stacks of thylakoids called grana form a third membrane layer.

Other organelles similar to mitochondria have arisen in other types of eukaryotes, but their roles differ. Hydrogenosomes are found in some anaerobic eukaryotes and serve as the location of anaerobic hydrogen production. Hydrogenosomes typically lack their own DNA and ribosomes. Kinetoplasts are a variation of the mitochondria found in some eukaryotic pathogens. In these organisms, each cell has a single, long, branched mitochondrion in which kinetoplast DNA, organized as multiple circular pieces of DNA, is found concentrated at one pole of the cell.

Note:

Mitochondria-Related Organelles in Protozoan Parasites

Many protozoans, including several protozoan parasites that cause infections in humans, can be identified by their unusual appearance. Distinguishing features may include complex cell morphologies, the presence of unique organelles, or the absence of common organelles. The protozoan parasites *Giardia lamblia* and *Trichomonas vaginalis* are two examples.

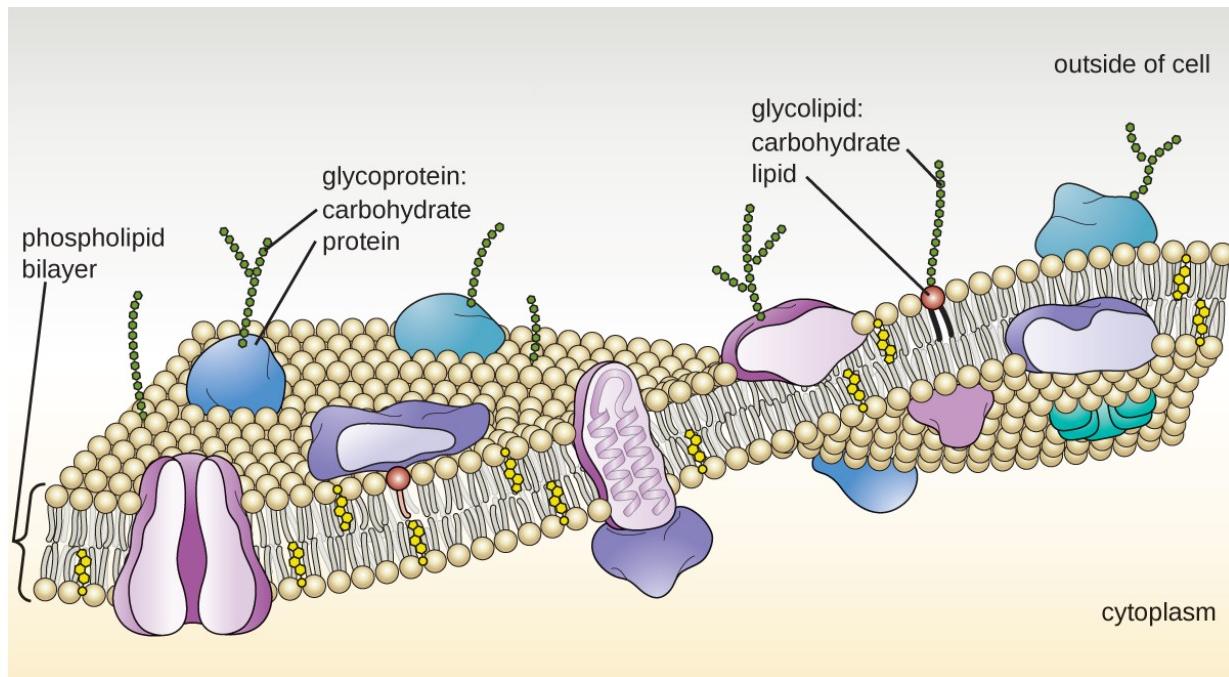
G. lamblia, a frequent cause of diarrhea in humans and many other animals, is an anaerobic parasite that possesses two nuclei and several flagella. Its Golgi apparatus and endoplasmic reticulum are greatly reduced, and it lacks mitochondria completely. However, it does have organelles known as mitosomes, double-membrane-bound organelles that appear to be severely reduced mitochondria. This has led scientists to believe that *G. lamblia*'s ancestors once possessed mitochondria that evolved to become mitosomes. *T. vaginalis*, which causes the sexually transmitted infection vaginitis, is another protozoan parasite that lacks conventional mitochondria. Instead, it possesses hydrogenosomes, mitochondrial-related, double-membrane-bound organelles that produce molecular

hydrogen used in cellular metabolism. Scientists believe that hydrogenosomes, like mitosomes, also evolved from mitochondria.[\[footnote\]](#)

N. Yarlett, J.H.P. Hackstein. "Hydrogenosomes: One Organelle, Multiple Origins." *BioScience* 55 no. 8 (2005):657–658.

Plasma Membrane

The plasma membrane of eukaryotic cells is similar in structure to the prokaryotic plasma membrane in that it is composed mainly of phospholipids forming a bilayer with embedded peripheral and integral proteins ([\[link\]](#)). These membrane components move within the plane of the membrane according to the fluid mosaic model. However, unlike the prokaryotic membrane, eukaryotic membranes contain sterols, including cholesterol, that alter membrane fluidity. Additionally, many eukaryotic cells contain some specialized lipids, including sphingolipids, which are thought to play a role in maintaining membrane stability as well as being involved in signal transduction pathways and cell-to-cell communication.

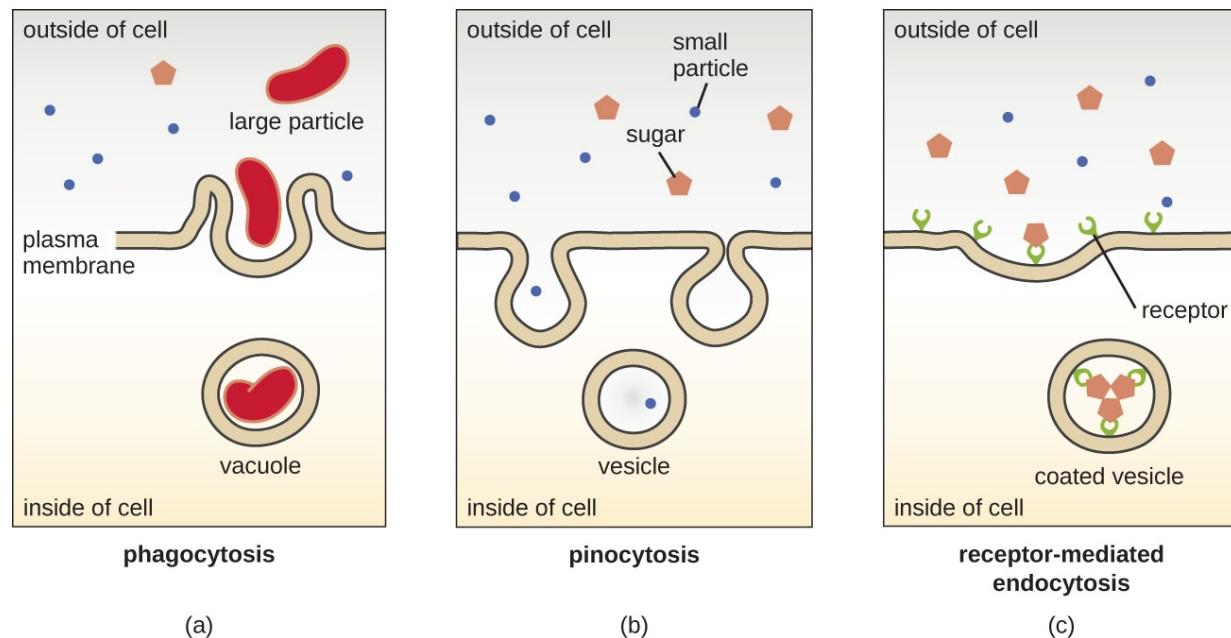


The eukaryotic plasma membrane is composed of a lipid bilayer with many embedded or associated proteins. It contains cholesterol for the maintenance of membrane, as well as glycoproteins and glycolipids that are important in the recognition other cells or pathogens.

Membrane Transport Mechanisms

The processes of simple diffusion, facilitated diffusion, and active transport are used in both eukaryotic and prokaryotic cells. However, eukaryotic cells also have the unique ability to perform various types of **endocytosis**, the uptake of matter through plasma membrane invagination and vacuole/vesicle formation ([\[link\]](#)). A type of endocytosis involving the engulfment of large particles through membrane invagination is called **phagocytosis**, which means “cell eating.” In phagocytosis, particles (or other cells) are enclosed in a pocket within the membrane, which then pinches off from the membrane to form a vacuole that completely surrounds the particle. Another type of endocytosis is called **pinocytosis**, which means “cell drinking.” In pinocytosis, small, dissolved materials and liquids are taken into the cell through small vesicles. Saprophytic fungi, for example, obtain their nutrients from dead and decaying matter largely through pinocytosis.

Receptor-mediated endocytosis is a type of endocytosis that is initiated by specific molecules called ligands when they bind to cell surface receptors on the membrane. Receptor-mediated endocytosis is the mechanism that peptide and amine-derived hormones use to enter cells and is also used by various viruses and bacteria for entry into host cells.



Three variations of endocytosis are shown. (a) In phagocytosis, the cell membrane surrounds the particle and pinches off to form an intracellular vacuole. (b) In pinocytosis, the cell membrane surrounds a small volume of fluid and pinches off, forming a vesicle. (c) In receptor-mediated endocytosis, the uptake of substances is targeted to a specific substance (a ligand) that binds at the receptor on the external cell membrane. (credit: modification of work by Mariana Ruiz Villarreal)

The process by which secretory vesicles release their contents to the cell's exterior is called **exocytosis**. Vesicles move toward the plasma membrane and then meld with the membrane, ejecting their contents out of the cell. Exocytosis is used by cells to remove waste products and may also be used to release chemical signals that can be taken up by other cells.

Cell Wall

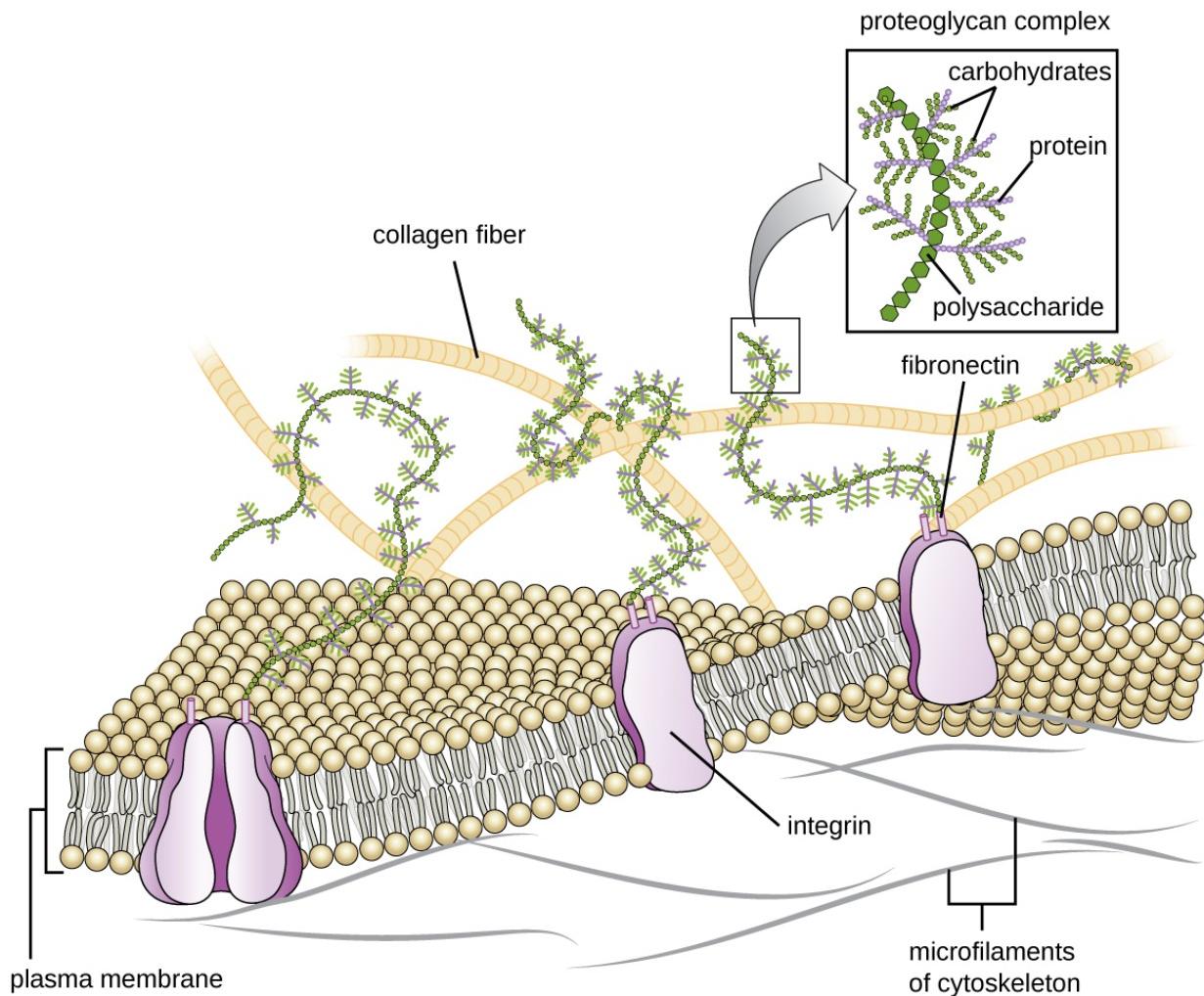
In addition to a plasma membrane, some eukaryotic cells have a cell wall. Cells of fungi, algae, plants, and even some protists have cell walls. Depending upon the type of eukaryotic cell, cell walls can be made of a wide range of materials, including cellulose (fungi and plants); biogenic silica, calcium carbonate, agar, and carrageenan (protists and algae); or chitin (fungi). In general, all cell walls provide structural stability for the cell and protection from environmental stresses such as desiccation, changes in osmotic pressure, and traumatic injury.[\[footnote\]](#)

M. Dudzick. "Protists." OpenStax CNX. November 27, 2013. <http://cnx.org/contents/f7048bb6-e462-459b-805c-ef291cf7049c@1>

Extracellular Matrix

Cells of animals and some protozoans do not have cell walls to help maintain shape and provide structural stability. Instead, these types of eukaryotic cells produce an **extracellular matrix** for this purpose. They secrete a sticky mass of carbohydrates and proteins into the spaces between adjacent cells ([\[link\]](#)). Some protein components assemble into a basement membrane to which the remaining extracellular matrix components adhere. Proteoglycans typically form the bulky mass of the extracellular matrix while fibrous proteins, like collagen, provide strength. Both proteoglycans and collagen are attached to fibronectin proteins, which, in turn, are attached to integrin proteins. These integrin proteins interact with transmembrane proteins in the plasma membranes of eukaryotic cells that lack cell walls.

In animal cells, the extracellular matrix allows cells within tissues to withstand external stresses and transmits signals from the outside of the cell to the inside. The amount of extracellular matrix is quite extensive in various types of connective tissues, and variations in the extracellular matrix can give different types of tissues their distinct properties. In addition, a host cell's extracellular matrix is often the site where microbial pathogens attach themselves to establish infection. For example, *Streptococcus pyogenes*, the bacterium that causes strep throat and various other infections, binds to fibronectin in the extracellular matrix of the cells lining the oropharynx (upper region of the throat).



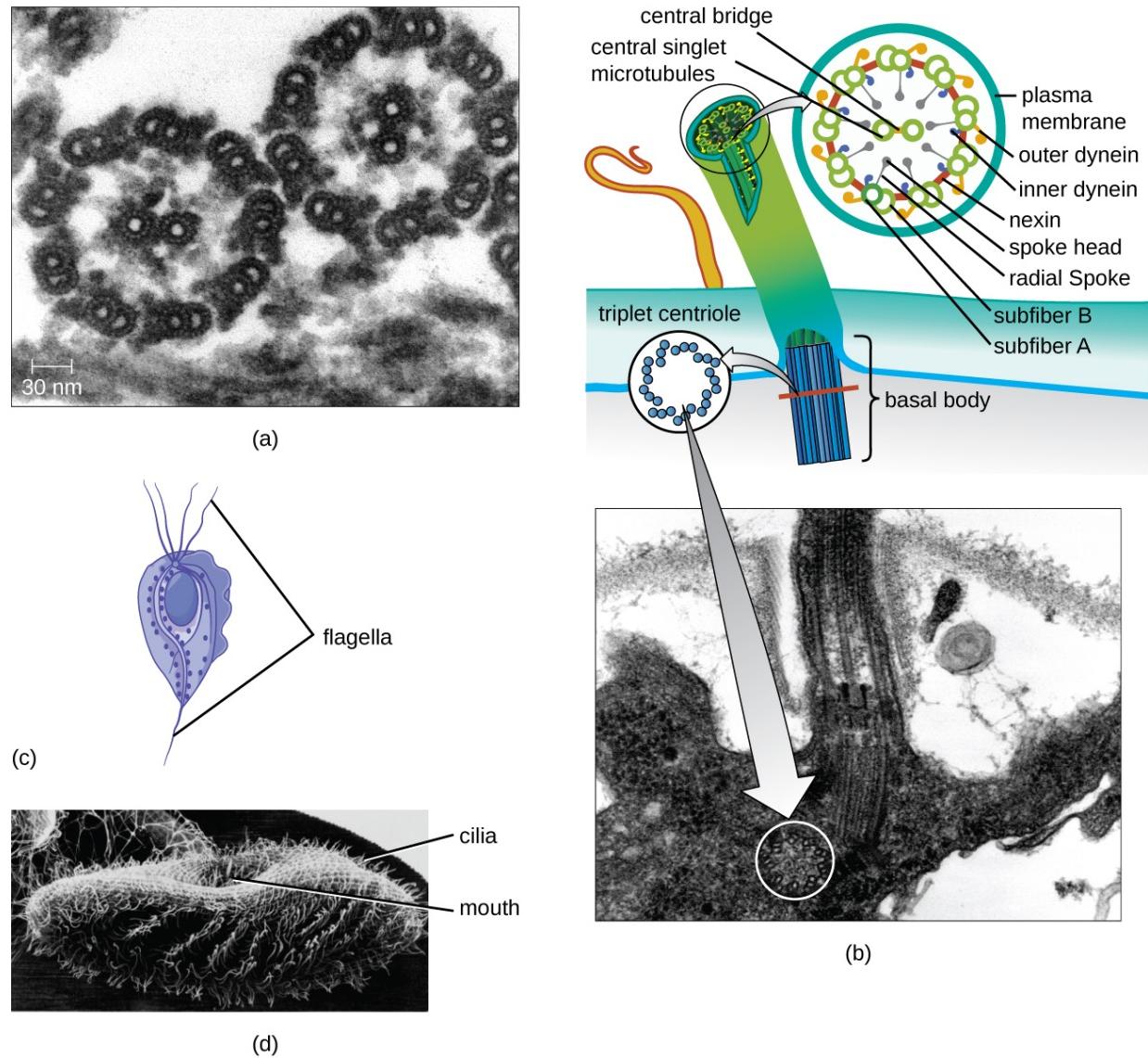
The extracellular matrix is composed of protein and carbohydrate components. It protects cells from physical stresses and transmits signals arriving at the outside edges of the tissue to cells deeper within the tissue.

Flagella and Cilia

Some eukaryotic cells use **flagella** for locomotion; however, eukaryotic flagella are structurally distinct from those found in prokaryotic cells. Whereas the prokaryotic flagellum is a stiff, rotating structure, a eukaryotic flagellum is more like a flexible whip composed of nine parallel pairs of microtubules surrounding a central pair of microtubules. This arrangement is referred to as a 9+2 array ([\[link\]](#)). The parallel microtubules use **dynein** motor proteins to move relative to each other, causing the flagellum to bend.

Cilia (singular: **cilium**) are a similar external structure found in some eukaryotic cells. Unique to eukaryotes, cilia are shorter than flagella and often cover the entire surface of a cell; however, they are structurally similar to flagella (a 9+2 array of microtubules) and use the same mechanism for movement. A structure called a **basal body** is found at the base of each cilium and flagellum. The

basal body, which attaches the cilium or flagellum to the cell, is composed of an array of triplet microtubules similar to that of a centriole but embedded in the plasma membrane. Because of their shorter length, cilia use a rapid, flexible, waving motion. In addition to motility, cilia may have other functions such as sweeping particles past or into cells. For example, ciliated protozoans use the sweeping of cilia to move food particles into their mouthparts, and ciliated cells in the mammalian respiratory tract beat in synchrony to sweep mucus and debris up and out of the lungs ([\[link\]](#)).



(a) Eukaryotic flagella and cilia are composed of a 9+2 array of microtubules, as seen in this transmission electron micrograph cross-section. (b) The sliding of these microtubules relative to each other causes a flagellum to bend. (c) An illustration of *Trichomonas vaginalis*, a flagellated protozoan parasite that causes vaginitis. (d) Many protozoans, like this *Paramecium*, have numerous cilia that aid in locomotion as well as in feeding. Note the mouth opening shown here.
 (credit d: modification of work by University of Vermont/National Institutes of Health)

Note:

- Explain how the cellular envelope of eukaryotic cells compares to that of prokaryotic cells.
- Explain the difference between eukaryotic and prokaryotic flagella.

Note:**Resolution**

Since amoxicillin has not resolved Barbara's case of pneumonia, the PA prescribes another antibiotic, azithromycin, which targets bacterial ribosomes rather than peptidoglycan. After taking the azithromycin as directed, Barbara's symptoms resolve and she finally begins to feel like herself again. Presuming no drug resistance to amoxicillin was involved, and given the effectiveness of azithromycin, the causative agent of Barbara's pneumonia is most likely *Mycoplasma pneumoniae*. Even though this bacterium is a prokaryotic cell, it is not inhibited by amoxicillin because it does not have a cell wall and, therefore, does not make peptidoglycan.

Go back to the [previous](#) Clinical Focus box.

Key Concepts and Summary

- Eukaryotic cells are defined by the presence of a **nucleus** containing the DNA genome and bound by a **nuclear membrane** (or **nuclear envelope**) composed of two lipid bilayers that regulate transport of materials into and out of the nucleus through nuclear pores.
- Eukaryotic cell morphologies vary greatly and may be maintained by various structures, including the cytoskeleton, the cell membrane, and/or the cell wall
- The **nucleolus**, located in the nucleus of eukaryotic cells, is the site of ribosomal synthesis and the first stages of ribosome assembly.
- Eukaryotic cells contain **80S ribosomes** in the rough endoplasmic reticulum (**membrane bound-ribosomes**) and cytoplasm (**free ribosomes**). They contain 70s ribosomes in mitochondria and chloroplasts.
- Eukaryotic cells have evolved an **endomembrane** system, containing membrane-bound organelles involved in transport. These include vesicles, the endoplasmic reticulum, and the Golgi apparatus.
- The **smooth endoplasmic reticulum** plays a role in lipid biosynthesis, carbohydrate metabolism, and detoxification of toxic compounds. The **rough endoplasmic reticulum** contains membrane-bound 80S ribosomes that synthesize proteins destined for the cell membrane
- The **Golgi apparatus** processes proteins and lipids, typically through the addition of sugar molecules, producing glycoproteins or glycolipids, components of the plasma membrane that are used in cell-to-cell communication.
- **Lysosomes** contain digestive enzymes that break down small particles ingested by **endocytosis**, large particles or cells ingested by **phagocytosis**, and damaged intracellular components.
- The **cytoskeleton**, composed of **microfilaments**, **intermediate filaments**, and **microtubules**, provides structural support in eukaryotic cells and serves as a network for transport of intracellular materials.

- **Centrosomes** are microtubule-organizing centers important in the formation of the mitotic spindle in mitosis.
- **Mitochondria** are the site of cellular respiration. They have two membranes: an outer membrane and an inner membrane with cristae. The mitochondrial matrix, within the inner membrane, contains the mitochondrial DNA, 70S ribosomes, and metabolic enzymes.
- The plasma membrane of eukaryotic cells is structurally similar to that found in prokaryotic cells, and membrane components move according to the fluid mosaic model. However, eukaryotic membranes contain sterols, which alter membrane fluidity, as well as glycoproteins and glycolipids, which help the cell recognize other cells and infectious particles.
- In addition to active transport and passive transport, eukaryotic cell membranes can take material into the cell via **endocytosis**, or expel matter from the cell via **exocytosis**.
- Cells of fungi, algae, plants, and some protists have a **cell wall**, whereas cells of animals and some protozoans have a sticky **extracellular matrix** that provides structural support and mediates cellular signaling.
- Eukaryotic flagella are structurally distinct from prokaryotic flagella but serve a similar purpose (locomotion). **Cilia** are structurally similar to eukaryotic flagella, but shorter; they may be used for locomotion, feeding, or movement of extracellular particles.

Multiple Choice

Exercise:

Problem: Which of the following organelles is not part of the endomembrane system?

- A. endoplasmic reticulum
- B. Golgi apparatus
- C. lysosome
- D. peroxisome

Solution:

D

Exercise:

Problem: Which type of cytoskeletal fiber is important in the formation of the nuclear lamina?

- A. microfilaments
- B. intermediate filaments
- C. microtubules
- D. fibronectin

Solution:

B

Exercise:

Problem: Sugar groups may be added to proteins in which of the following?

-
- A. smooth endoplasmic reticulum
 - B. rough endoplasmic reticulum
 - C. Golgi apparatus
 - D. lysosome
-

Solution:

C

Exercise:

Problem:

Which of the following structures of a eukaryotic cell is not likely derived from endosymbiotic bacterium?

- A. mitochondrial DNA
 - B. mitochondrial ribosomes
 - C. inner membrane
 - D. outer membrane
-

Solution:

D

Exercise:

Problem:

Which type of nutrient uptake involves the engulfment of small dissolved molecules into vesicles?

- A. active transport
 - B. pinocytosis
 - C. receptor-mediated endocytosis
 - D. facilitated diffusion
-

Solution:

B

Exercise:

Problem: Which of the following is not composed of microtubules?

- A. desmosomes
- B. centrioles
- C. eukaryotic flagella
- D. eukaryotic cilia

Solution:

A

True/False**Exercise:****Problem:**

Mitochondria in eukaryotic cells contain ribosomes that are structurally similar to those found in prokaryotic cells.

Solution:

True

Fill in the Blank**Exercise:****Problem:**

Peroxisomes typically produce _____, a harsh chemical that helps break down molecules.

Solution:

hydrogen peroxide

Exercise:

Problem: Microfilaments are composed of _____ monomers.

Solution:

actin

Short Answer**Exercise:****Problem:**

What existing evidence supports the theory that mitochondria are of prokaryotic origin?

Exercise:

Problem: Why do eukaryotic cells require an endomembrane system?

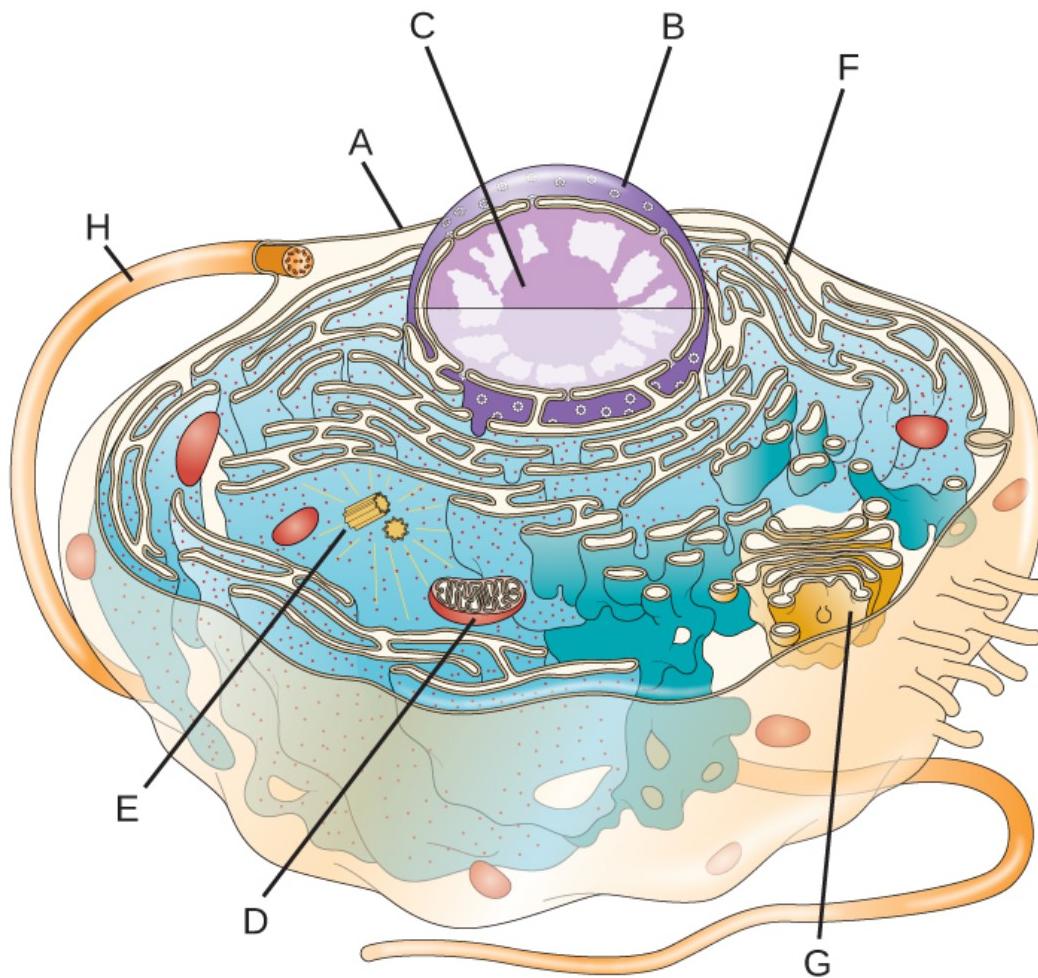
Exercise:

Problem: Name at least two ways that prokaryotic flagella are different from eukaryotic flagella.

Critical Thinking

Exercise:

Problem: Label the lettered parts of this eukaryotic cell.



Exercise:

Problem:

How are peroxisomes more like mitochondria than like the membrane-bound organelles of the endomembrane system? How do they differ from mitochondria?

Exercise:

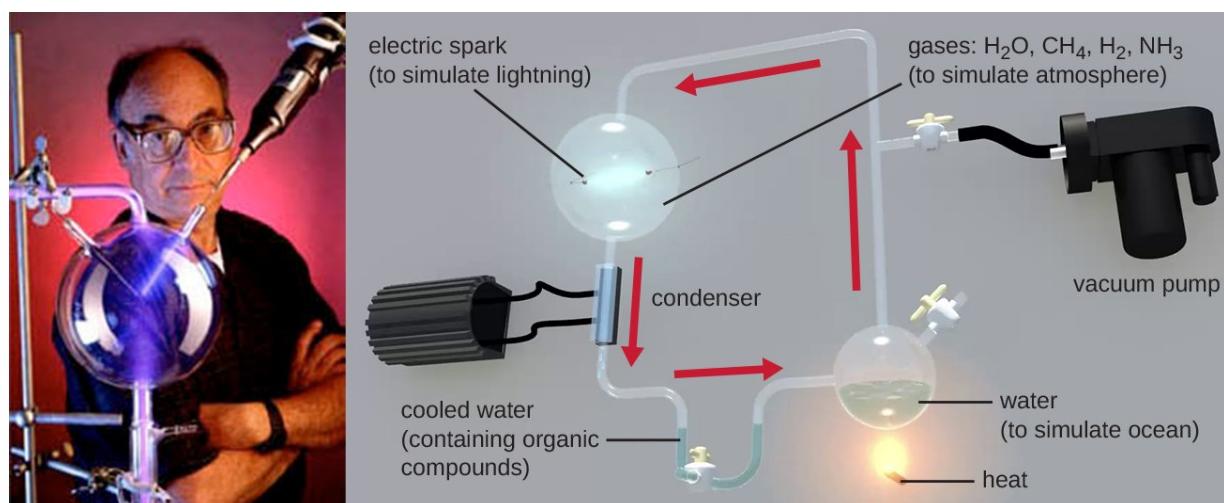
Problem: Why must the functions of both lysosomes and peroxisomes be compartmentalized?

Microbial Biochemistry - Introduction

class="introduction"

Scientist
Stanley
Miller
(pictured)
and Harold
Urey
demonstrated
that organic
compounds
may have
originated
naturally
from
inorganic
matter. The
Miller-Urey
experiment
illustrated
here
simulated the
effects of
lightning on
chemical
compounds
found in the
earth's early
atmosphere.
The resulting
reactions
yielded
amino acids,
the chemical
building
blocks of

proteins.
(credit
“photo”:
modification
of work by
NASA;
credit
“illustration”
:
modification
of work by
Courtney
Harrington)



The earth is estimated to be 4.6 billion years old, but for the first 2 billion years, the atmosphere lacked oxygen, without which the earth could not support life as we know it. One hypothesis about how life emerged on earth involves the concept of a “primordial soup.” This idea proposes that life began in a body of water when metals and gases from the atmosphere combined with a source of energy, such as lightning or ultraviolet light, to form the carbon compounds that are the chemical building blocks of life. In 1952, Stanley Miller (1930–2007), a graduate student at the University of Chicago, and his professor Harold Urey (1893–1981), set out to confirm this hypothesis in a now-famous experiment. Miller and Urey combined

what they believed to be the major components of the earth's early atmosphere—water (H_2O), methane (CH_4), hydrogen (H_2), and ammonia (NH_3)—and sealed them in a sterile flask. Next, they heated the flask to produce water vapor and passed electric sparks through the mixture to mimic lightning in the atmosphere ([\[link\]](#)). When they analyzed the contents of the flask a week later, they found amino acids, the structural units of proteins—molecules essential to the function of all organisms.

Organic Molecules

LEARNING OBJECTIVES

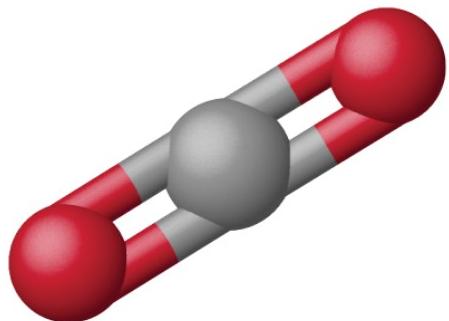
- Identify common elements and structures found in organic molecules
- Identify examples of functional groups
- Describe the role of functional groups in synthesizing polymers

Biochemistry is the discipline that studies the chemistry of life, and its objective is to explain form and function based on chemical principles. Organic chemistry is the discipline devoted to the study of carbon-based chemistry, which is the foundation for the study of biomolecules and the discipline of biochemistry. Both biochemistry and organic chemistry are based on the concepts of general chemistry, some of which are presented in [Appendix A](#).

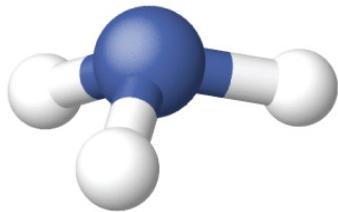
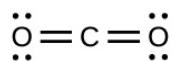
Elements in Living Cells

The most abundant element in cells is hydrogen (H), followed by carbon (C), oxygen (O), nitrogen (N), phosphorous (P), and sulfur (S). We call these elements **macronutrients**, and they account for about 99% of the dry weight of cells. Some elements, such as sodium (Na), potassium (K), magnesium (Mg), zinc (Zn), iron (Fe), calcium (Ca), molybdenum (Mo), copper (Cu), cobalt (Co), manganese (Mn), or vanadium (Va), are required by some cells in very small amounts and are called **micronutrients** or **trace elements**. All of these elements are essential to the function of many biochemical reactions, and, therefore, are essential to life.

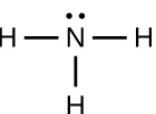
The four most abundant elements in living matter (C, N, O, and H) have low atomic numbers and are thus light elements capable of forming strong bonds with other atoms to produce molecules ([\[link\]](#)). Carbon forms four chemical bonds, whereas nitrogen forms three, oxygen forms two, and hydrogen forms one. When bonded together within molecules, oxygen, sulfur, and nitrogen often have one or more “lone pairs” of electrons that play important roles in determining many of the molecules’ physical and chemical properties (see [Appendix A](#)). These traits in combination permit the formation of a vast number of diverse molecular species necessary to form the structures and enable the functions of living organisms.



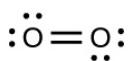
carbon dioxide
 CO_2



ammonia
 NH_3



oxygen
 O_2



Some common molecules include carbon dioxide, ammonia, and oxygen, which consist of combinations of oxygen atoms (red spheres), carbon atoms (gray spheres), hydrogen atoms (white spheres), or nitrogen atoms (blue spheres).

Living organisms contain inorganic compounds (mainly water and salts; see [Appendix A](#)) and organic molecules. Organic molecules contain carbon; inorganic compounds do not. Carbon oxides and carbonates are exceptions; they contain carbon but are considered inorganic because they do not contain hydrogen. The atoms of an **organic molecule** are typically organized around chains of carbon atoms.

Inorganic compounds make up 1%–1.5% of a living cell’s mass. They are small, simple compounds that play important roles in the cell, although they do not form cell structures. Most of the carbon found in organic molecules originates from inorganic carbon sources such as carbon dioxide captured via carbon fixation by microorganisms.

Note:

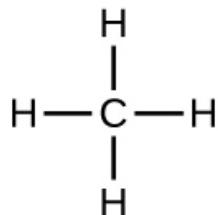
- Describe the most abundant elements in nature.
- What are the differences between organic and inorganic molecules?

Organic Molecules and Isomerism

Organic molecules in organisms are generally larger and more complex than inorganic molecules. Their carbon skeletons are held together by covalent bonds. They form the cells of

an organism and perform the chemical reactions that facilitate life. All of these molecules, called **biomolecules** because they are part of living matter, contain carbon, which is the building block of life. Carbon is a very unique element in that it has four valence electrons in its outer orbitals and can form four single covalent bonds with up to four other atoms at the same time (see [Appendix A](#)). These atoms are usually oxygen, hydrogen, nitrogen, sulfur, phosphorous, and carbon itself; the simplest organic compound is methane, in which carbon binds only to hydrogen ([\[link\]](#)).

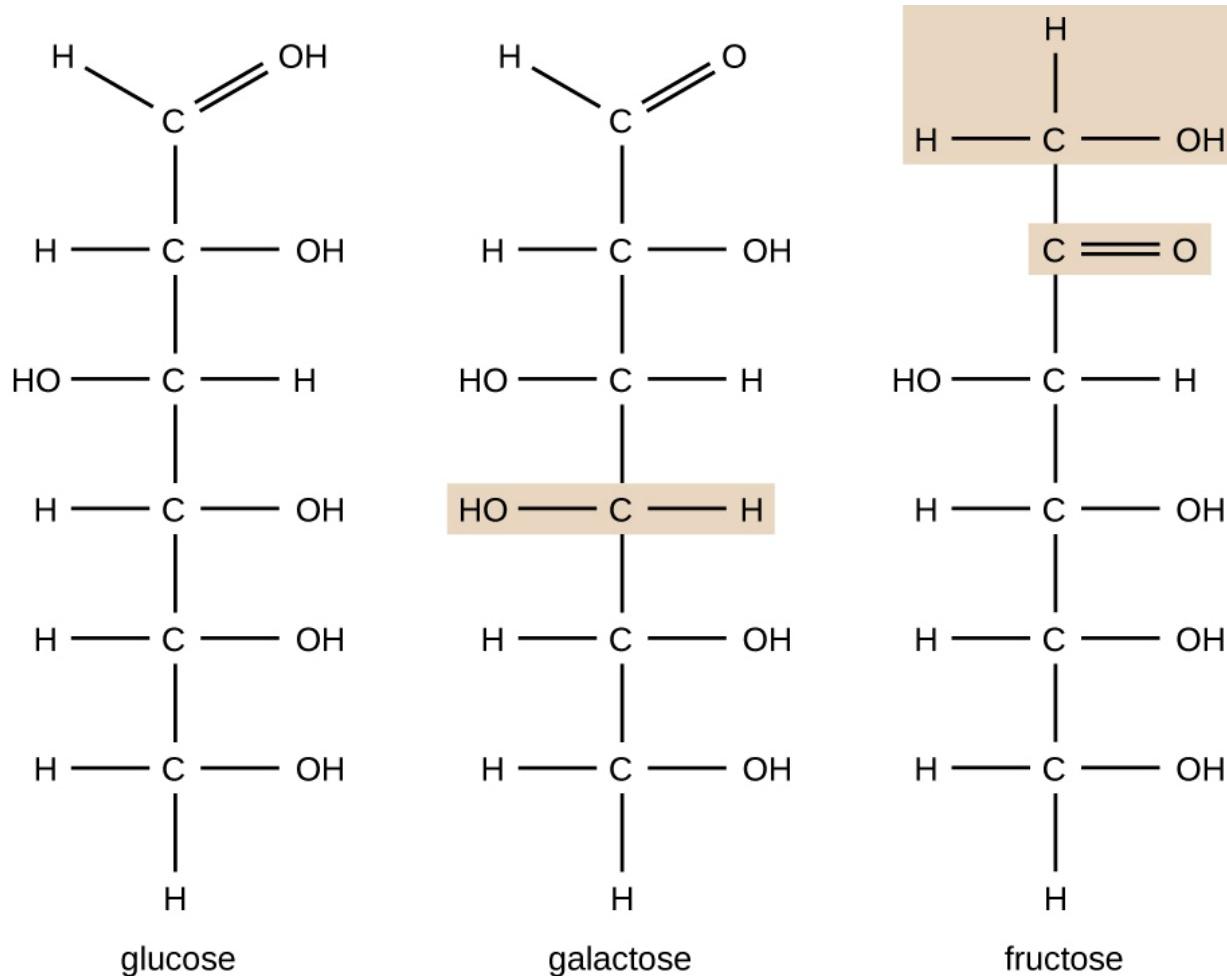
As a result of carbon's unique combination of size and bonding properties, carbon atoms can bind together in large numbers, thus producing a chain or **carbon skeleton**. The carbon skeleton of organic molecules can be straight, branched, or ring shaped (cyclic). Organic molecules are built on chains of carbon atoms of varying lengths; most are typically very long, which allows for a huge number and variety of compounds. No other element has the ability to form so many different molecules of so many different sizes and shapes.



A carbon atom can bond with up to four other atoms. The simplest organic molecule is methane (CH_4), depicted here.

Molecules with the same atomic makeup but different structural arrangement of atoms are called **isomers**. The concept of isomerism is very important in chemistry because the structure of a molecule is always directly related to its function. Slight changes in the structural arrangements of atoms in a molecule may lead to very different properties. Chemists represent molecules by their **structural formula**, which is a graphic representation of the molecular structure, showing how the atoms are arranged. Compounds that have identical molecular

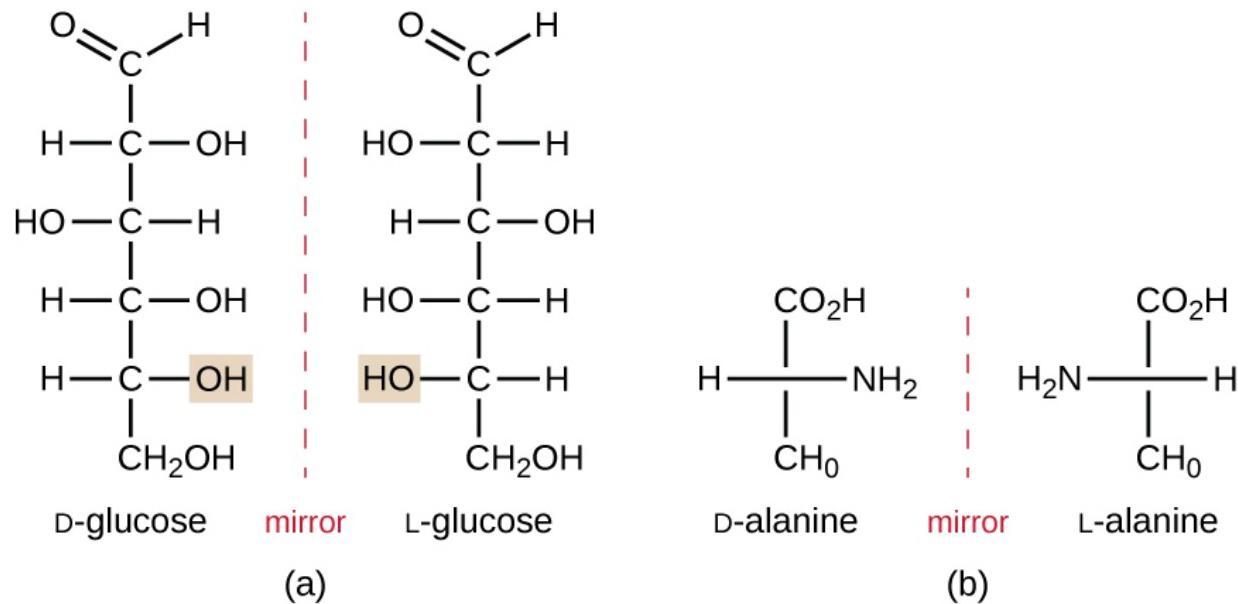
formulas but differ in the bonding sequence of the atoms are called **structural isomers**. The monosaccharides glucose, galactose, and fructose all have the same molecular formula, $C_6H_{12}O_6$, but we can see from [\[link\]](#) that the atoms are bonded together differently.



Glucose, galactose, and fructose have the same chemical formula ($C_6H_{12}O_6$), but these structural isomers differ in their physical and chemical properties.

Isomers that differ in the spatial arrangements of atoms are called **stereoisomers**; one unique type is **enantiomers**. The properties of enantiomers were originally discovered by Louis Pasteur in 1848 while using a microscope to analyze crystallized fermentation products of wine. Enantiomers are molecules that have the characteristic of **chirality**, in which their structures are nonsuperimposable mirror images of each other. Chirality is an important characteristic in many biologically important molecules, as illustrated by the examples of structural differences in the enantiomeric forms of the monosaccharide glucose or the amino acid alanine ([\[link\]](#)).

Many organisms are only able to use one enantiomeric form of certain types of molecules as nutrients and as building blocks to make structures within a cell. Some enantiomeric forms of amino acids have distinctly different tastes and smells when consumed as food. For example, L-aspartame, commonly called aspartame, tastes sweet, whereas D-aspartame is tasteless. Drug enantiomers can have very different pharmacologic affects. For example, the compound methorphan exists as two enantiomers, one of which acts as an antitussive (*dextromethorphan*, a cough suppressant), whereas the other acts as an analgesic (*levomethorphan*, a drug similar in effect to codeine).



Enantiomers are stereoisomers that exhibit chirality. Their chemical structures are nonsuperimposable mirror images of each other. (a) D-glucose and L-glucose are monosaccharides that are enantiomers. (b) The enantiomers D-alanine and L-alanine are enantiomers found in bacterial cell walls and human cells, respectively.

Enantiomers are also called optical isomers because they can rotate the plane of polarized light. Some of the crystals Pasteur observed from wine fermentation rotated light clockwise whereas others rotated the light counterclockwise. Today, we denote enantiomers that rotate polarized light clockwise (+) as *d* forms, and the mirror image of the same molecule that rotates polarized light counterclockwise (−) as the *l* form. The *d* and *l* labels are derived from the Latin words *dexter* (on the right) and *laevus* (on the left), respectively. These two different optical isomers often have very different biological properties and activities. Certain species of molds, yeast, and bacteria, such as *Rhizopus*, *Yarrowia*, and *Lactobacillus* spp., respectively, can only metabolize one type of optical isomer; the opposite isomer is not suitable as a source of nutrients. Another important reason to be aware of optical isomers is the therapeutic use of

these types of chemicals for drug treatment, because some microorganisms can only be affected by one specific optical isomer.

Note:

- We say that life is carbon based. What makes carbon so suitable to be part of all the macromolecules of living organisms?

Biologically Significant Functional Groups

In addition to containing carbon atoms, biomolecules also contain **functional groups**—groups of atoms within molecules that are categorized by their specific chemical composition and the chemical reactions they perform, regardless of the molecule in which the group is found. Some of the most common functional groups are listed in [link]. In the formulas, the symbol R stands for “residue” and represents the remainder of the molecule. R might symbolize just a single hydrogen atom or it may represent a group of many atoms. Notice that some functional groups are relatively simple, consisting of just one or two atoms, while some comprise two of these simpler functional groups. For example, a carbonyl group is a functional group composed of a carbon atom double bonded to an oxygen atom: C=O. It is present in several classes of organic compounds as part of larger functional groups such as ketones, aldehydes, carboxylic acids, and amides. In ketones, the carbonyl is present as an internal group, whereas in aldehydes it is a terminal group.

Common Functional Groups Found in Biomolecules		
Name	Functional Group	Compounds
Aldehyde	$\text{R}-\text{C}(=\text{O})-\text{H}$	Carbohydrates
Amide	$\text{R}-\text{C}(=\text{O})-\text{N}(\text{H})-\text{R}'$	Proteins
Amino	$\text{R}-\text{NH}_2$	Amino acids, proteins
Carbonyl	$\text{R}-\text{C}(=\text{O})-\text{R}'$	Ketones, aldehydes, carboxylic acids, amides
Carboxylic acid	$\text{R}-\text{C}(=\text{O})-\text{O}-\text{H}$	Amino acids, proteins, fatty acids
Ester	$\text{R}-\text{C}(=\text{O})-\text{O}-\text{R}'$	Lipids, nucleic acids
Ether	$\text{R}-\text{O}-\text{R}'$	Disaccharides, polysaccharides, lipids
Hydroxyl	$\text{R}-\text{O}-\text{H}$	Alcohols, monosaccharides, amino acids, nucleic acids
Ketone	$\text{R}-\text{C}(=\text{O})-\text{R}'$	Carbohydrates
Methyl	$\text{R}-\text{CH}_3$	Methylated compounds such as methyl alcohols and methyl esters
Phosphate	$\text{R}-\text{PO}_3\text{H}_2$	Nucleic acids, phospholipids, ATP
Sulfhydryl	$\text{R}-\text{S}-\text{H}$	Amino acids, proteins

*Functional groups are represented in pink. Ketone and aldehyde both contain a carbonyl group, highlighted in blue.

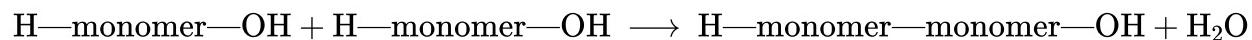
Macromolecules

Carbon chains form the skeletons of most organic molecules. Functional groups combine with the chain to form biomolecules. Because these biomolecules are typically large, we call them **macromolecules**. Many biologically relevant macromolecules are formed by linking together a great number of identical, or very similar, smaller organic molecules. The smaller molecules act as building blocks and are called **monomers**, and the macromolecules that result from their

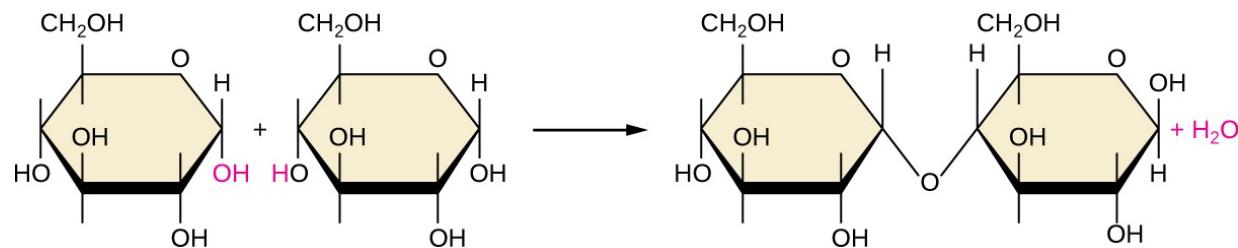
linkage are called **polymers**. Cells and cell structures include four main groups of carbon-containing macromolecules: polysaccharides, proteins, lipids, and nucleic acids. The first three groups of molecules will be studied throughout this chapter. The biochemistry of nucleic acids will be discussed in [Biochemistry of the Genome](#).

Of the many possible ways that monomers may be combined to yield polymers, one common approach encountered in the formation of biological macromolecules is **dehydration synthesis**. In this chemical reaction, monomer molecules bind end to end in a process that results in the formation of water molecules as a byproduct:

Equation:



[\[link\]](#) shows dehydration synthesis of glucose binding together to form maltose and a water molecule. [\[link\]](#) summarizes macromolecules and some of their functions.



In this dehydration synthesis reaction, two molecules of glucose are linked together to form maltose. In the process, a water molecule is formed.

Some Functions of Macromolecules

Macromolecule	Functions
Carbohydrates	Energy storage, receptors, food, structural role in plants, fungal cell walls, exoskeletons of insects
Lipids	Energy storage, membrane structure, insulation, hormones, pigments

Some Functions of Macromolecules

Macromolecule	Functions
Nucleic acids	Storage and transfer of genetic information
Proteins	Enzymes, structure, receptors, transport, structural role in the cytoskeleton of a cell and the extracellular matrix

Note:

- What is the byproduct of a dehydration synthesis reaction?

Key Concepts and Summary

- The most abundant elements in cells are hydrogen, carbon, oxygen, nitrogen, phosphorus, and sulfur.
- Life is carbon based. Each carbon atom can bind to another one producing a **carbon skeleton** that can be straight, branched, or ring shaped.
- The same numbers and types of atoms may bond together in different ways to yield different molecules called **isomers**. Isomers may differ in the bonding sequence of their atoms (**structural isomers**) or in the spatial arrangement of atoms whose bonding sequences are the same (**stereoisomers**), and their physical and chemical properties may vary slightly or drastically.
- **Functional groups** confer specific chemical properties to molecules bearing them. Common functional groups in biomolecules are hydroxyl, methyl, carbonyl, carboxyl, amino, phosphate, and sulfhydryl.
- **Macromolecules** are **polymers** assembled from individual units, the **monomers**, which bind together like building blocks. Many biologically significant macromolecules are formed by **dehydration synthesis**, a process in which monomers bind together by combining their functional groups and generating water molecules as byproducts.

Short Answer

Exercise:

Problem:

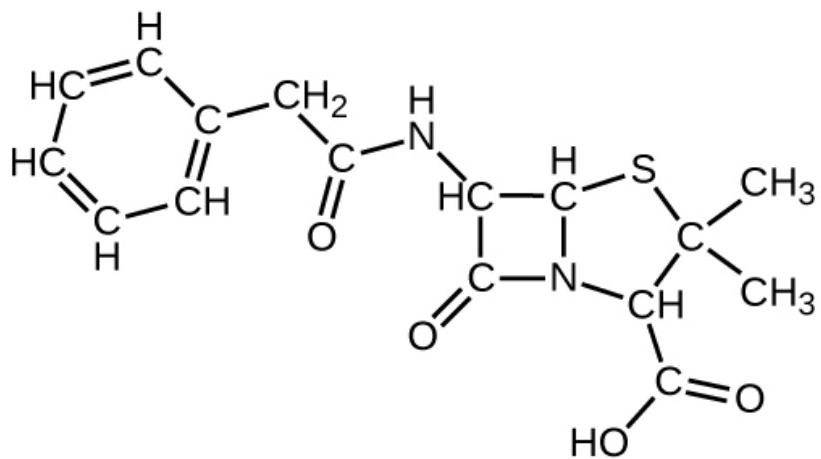
Why are carbon, nitrogen, oxygen, and hydrogen the most abundant elements in living matter and, therefore, considered macronutrients?

Critical Thinking

Exercise:

Problem:

The structural formula shown corresponds to penicillin G, a narrow-spectrum antibiotic that is given intravenously or intramuscularly as a treatment for several bacterial diseases. The antibiotic is produced by fungi of the genus *Penicillium*. (a) Identify three major functional groups in this molecule that each comprise two simpler functional groups. (b) Name the two simpler functional groups composing each of the major functional groups identified in (a).



Carbohydrates

LEARNING OBJECTIVES

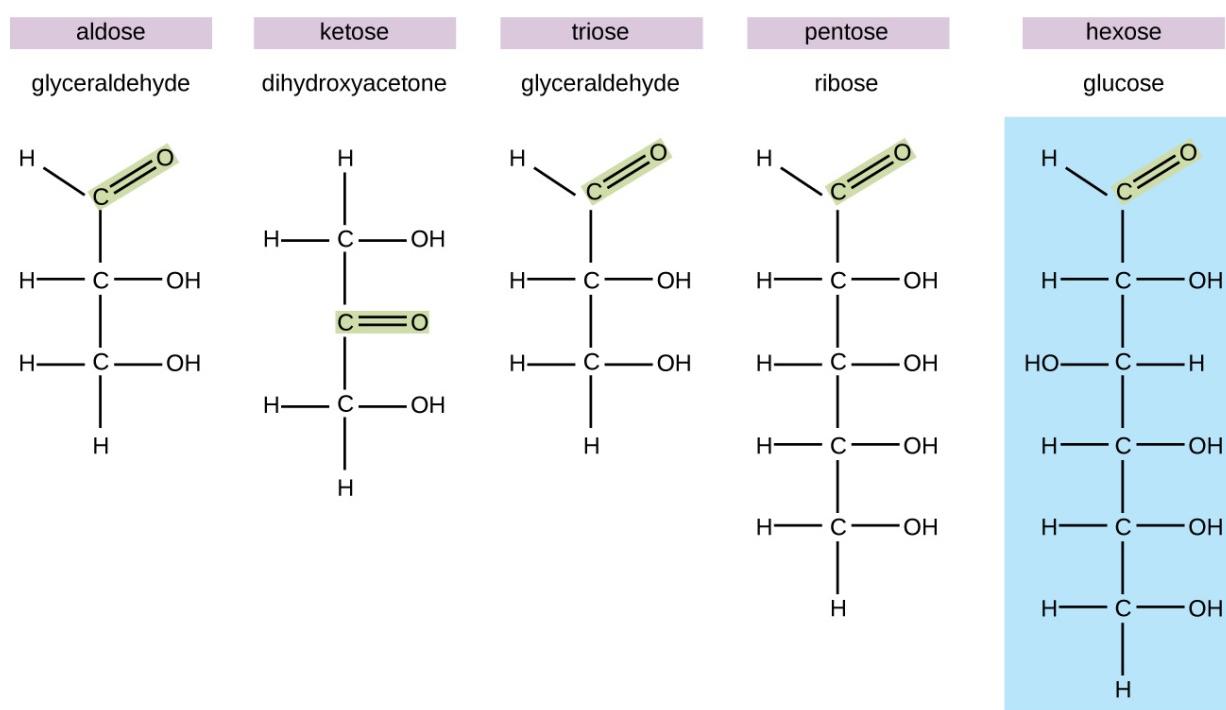
- Give examples of monosaccharides and polysaccharides
- Describe the function of monosaccharides and polysaccharides within a cell

The most abundant biomolecules on earth are **carbohydrates**. From a chemical viewpoint, carbohydrates are primarily a combination of carbon and water, and many of them have the empirical formula $(CH_2O)_n$, where n is the number of repeated units. This view represents these molecules simply as “hydrated” carbon atom chains in which water molecules attach to each carbon atom, leading to the term “carbohydrates.” Although all carbohydrates contain carbon, hydrogen, and oxygen, there are some that also contain nitrogen, phosphorus, and/or sulfur. Carbohydrates have myriad different functions. They are abundant in terrestrial ecosystems, many forms of which we use as food sources. These molecules are also vital parts of macromolecular structures that store and transmit genetic information (i.e., DNA and RNA). They are the basis of biological polymers that impart strength to various structural components of organisms (e.g., cellulose and chitin), and they are the primary source of energy storage in the form of starch and glycogen.

Monosaccharides: The Sweet Ones

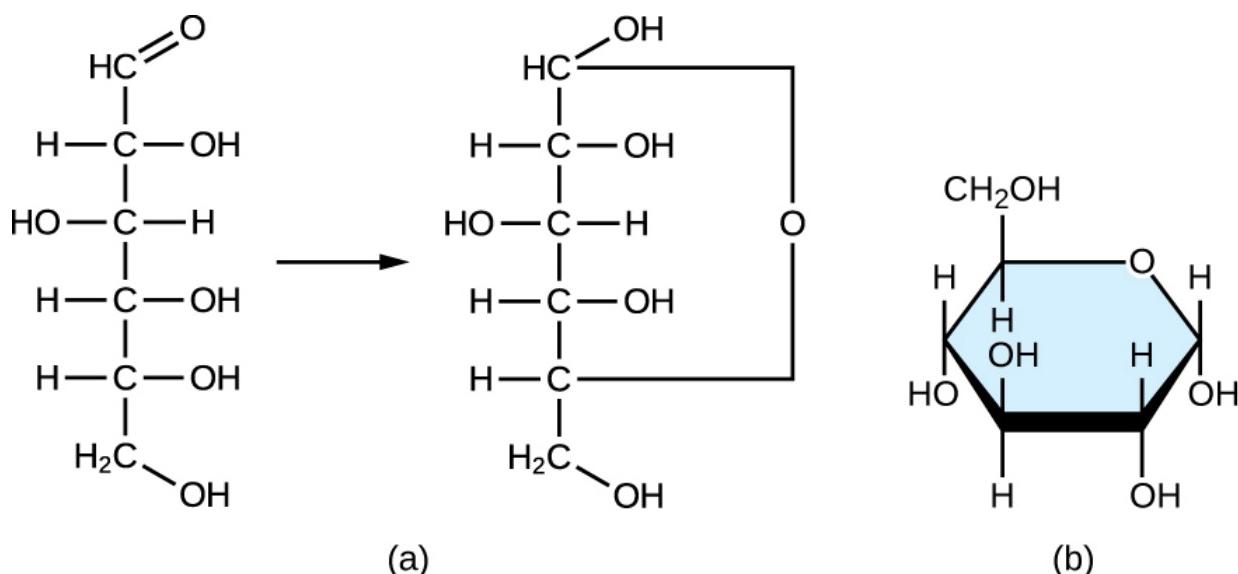
In biochemistry, carbohydrates are often called **saccharides**, from the Greek *sakcharon*, meaning sugar, although not all the saccharides are sweet. The simplest carbohydrates are called **monosaccharides**, or simple sugars. They are the building blocks (monomers) for the synthesis of polymers or complex carbohydrates, as will be discussed further in this section. Monosaccharides are classified based on the number of carbons in the molecule. General categories are identified using a prefix that indicates the number of carbons and the suffix *-ose*, which indicates a saccharide; for example, triose (three carbons), tetrose (four carbons), pentose (five carbons), and hexose (six carbons) ([\[link\]](#)). The hexose D-glucose is the most abundant monosaccharide in nature. Other very common and abundant hexose monosaccharides are galactose, used to make the disaccharide milk sugar lactose, and the fruit sugar fructose.

Monosaccharides



Monosaccharides are classified based on the position of the carbonyl group and the number of carbons in the backbone.

Monosaccharides of four or more carbon atoms are typically more stable when they adopt cyclic, or ring, structures. These ring structures result from a chemical reaction between functional groups on opposite ends of the sugar's flexible carbon chain, namely the carbonyl group and a relatively distant hydroxyl group. Glucose, for example, forms a six-membered ring ([\[link\]](#)).



(a) A linear monosaccharide (glucose in this case) forms a cyclic structure. (b) This illustration shows a more realistic depiction of the cyclic monosaccharide structure. Note in these cyclic structural diagrams, the carbon atoms composing the ring are not explicitly shown.

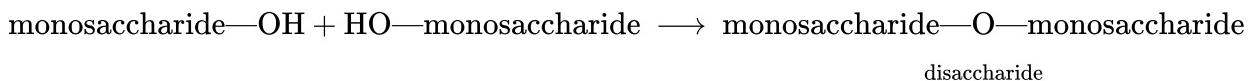
Note:

- Why do monosaccharides form ring structures?

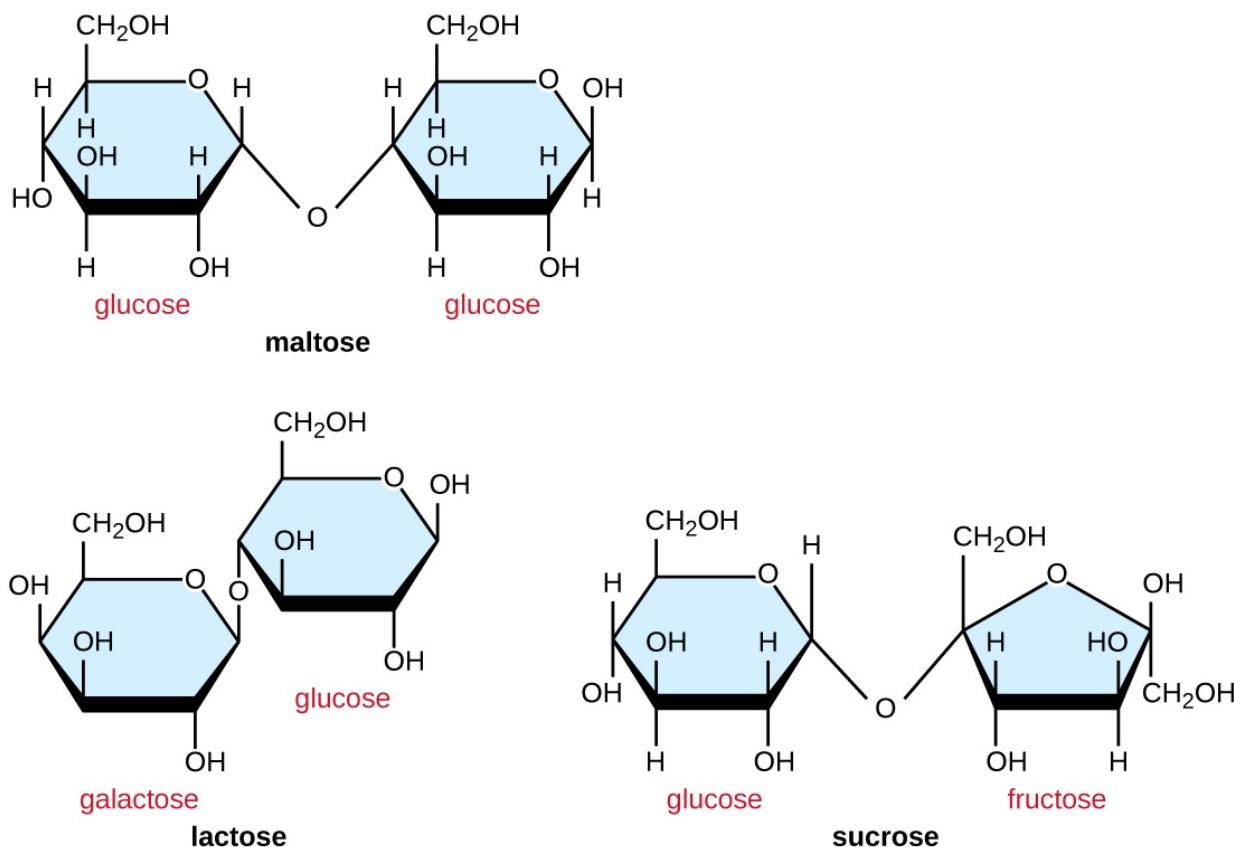
Disaccharides

Two monosaccharide molecules may chemically bond to form a **disaccharide**. The name given to the covalent bond between the two monosaccharides is a **glycosidic bond**. Glycosidic bonds form between hydroxyl groups of the two saccharide molecules, an example of the dehydration synthesis described in the previous section of this chapter:

Equation:



Common disaccharides are the grain sugar maltose, made of two glucose molecules; the milk sugar lactose, made of a galactose and a glucose molecule; and the table sugar sucrose, made of a glucose and a fructose molecule ([\[link\]](#)).



Common disaccharides include maltose, lactose, and sucrose.

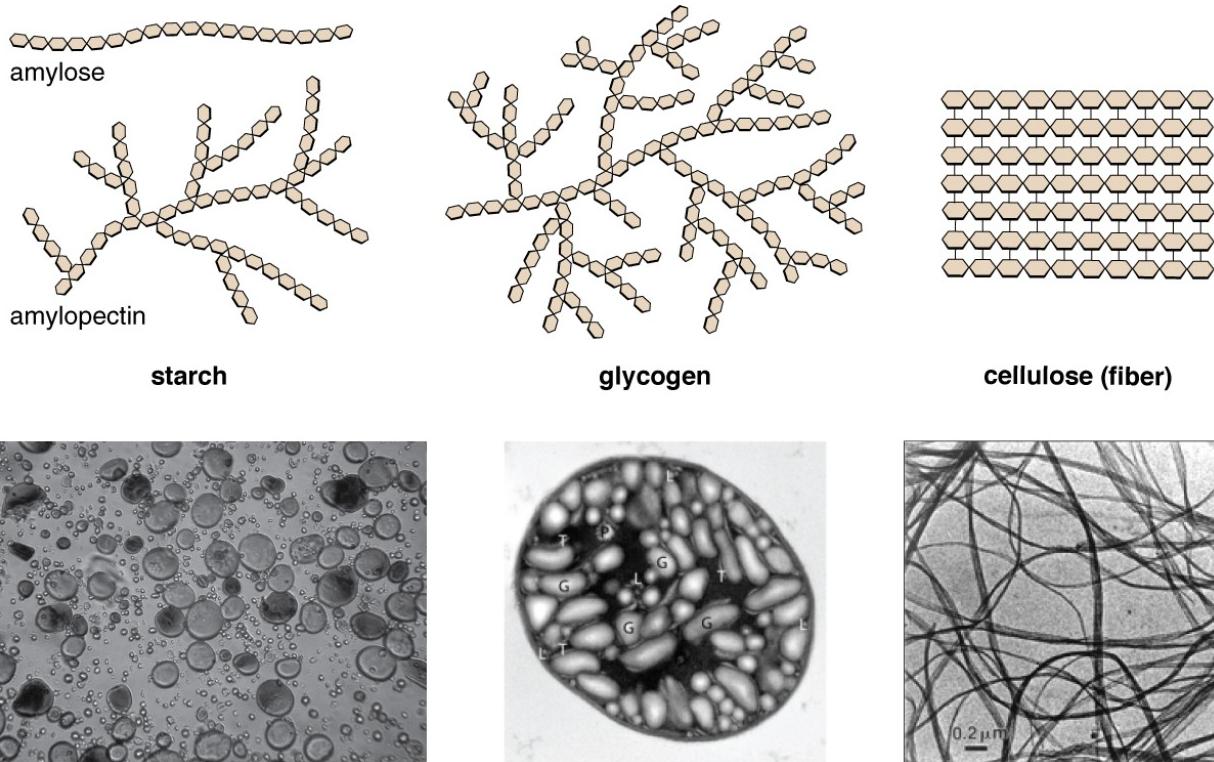
Polysaccharides

Polysaccharides, also called glycans, are large polymers composed of hundreds of monosaccharide monomers. Unlike mono- and disaccharides, **polysaccharides** are not sweet and, in general, they are not soluble in water. Like disaccharides, the monomeric units of polysaccharides are linked together by glycosidic bonds.

Polysaccharides are very diverse in their structure. Three of the most biologically important polysaccharides—**starch**, **glycogen**, and **cellulose**—are all composed of repetitive glucose units, although they differ in their structure ([\[link\]](#)). Cellulose consists of a linear chain of glucose molecules and is a common structural component of cell walls in plants and other organisms. Glycogen and starch are branched polymers; glycogen is the primary energy-storage molecule in animals and bacteria, whereas plants primarily store energy in starch. The orientation of the glycosidic linkages in these three polymers is different as well and, as a consequence, linear and branched macromolecules have different properties.

Modified glucose molecules can be fundamental components of other structural polysaccharides. Examples of these types of structural polysaccharides are N-acetyl glucosamine (NAG) and N-acetyl

muramic acid (NAM) found in bacterial cell wall peptidoglycan. Polymers of NAG form chitin, which is found in fungal cell walls and in the exoskeleton of insects.



Starch, glycogen, and cellulose are three of the most important polysaccharides. In the top row, hexagons represent individual glucose molecules. Micrographs (bottom row) show wheat starch granules stained with iodine (left), glycogen granules (G) inside the cell of a cyanobacterium (middle), and bacterial cellulose fibers (right). (credit “iodine granules”: modification of work by Kiselov Yuri; credit “glycogen granules”: modification of work by Stöckel J, Elvitigala TR, Liberton M, Pakrasi HB; credit “cellulose”: modification of work by American Society for Microbiology)

Note:

- What are the most biologically important polysaccharides and why are they important?

Key Concepts and Summary

- **Carbohydrates**, the most abundant biomolecules on earth, are widely used by organisms for structural and energy-storage purposes.
- Carbohydrates include individual sugar molecules (**monosaccharides**) as well as two or more molecules chemically linked by **glycosidic bonds**. **Monosaccharides** are classified based on the number of carbons the molecule has trioses (3 C), tetroses (4 C), pentoses (5 C), and hexoses (6 C). They are the building blocks for the synthesis of polymers or complex carbohydrates.
- **Disaccharides** such as sucrose, lactose, and maltose are molecules composed of two monosaccharides linked together by a glycosidic bond.
- **Polysaccharides**, or **glycans**, are polymers composed of hundreds of monosaccharide monomers linked together by glycosidic bonds. The energy-storage polymers **starch** and **glycogen** are examples of polysaccharides and are all composed of branched chains of glucose molecules.
- The polysaccharide **cellulose** is a common structural component of the cell walls of organisms. Other structural polysaccharides, such as N-acetyl glucosamine (NAG) and N-acetyl muramic acid (NAM), incorporate modified glucose molecules and are used in the construction of peptidoglycan or chitin.

Lipids

LEARNING OBJECTIVES

Describe the chemical composition of lipids

- Describe the unique characteristics and diverse structures of lipids
- Compare and contrast triacylglycerides (triglycerides) and phospholipids.
- Describe how phospholipids are used to construct biological membranes.
- Discuss the importance of lipids in bacterial metabolism

Although they are composed primarily of carbon and hydrogen, **lipid** molecules may also contain oxygen, nitrogen, sulfur, and phosphorous. Lipids serve numerous and diverse purposes in the structure and functions of organisms. They can be a source of nutrients, a storage form for carbon, energy-storage molecules, or structural components of membranes and hormones. Lipids comprise a broad class of many chemically distinct compounds, the most common of which are discussed in this section.

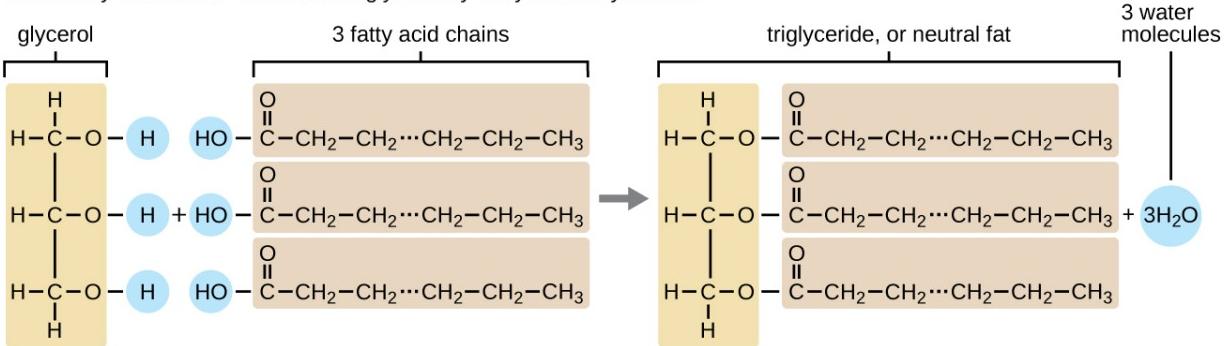
Fatty Acids and Triacylglycerides

The **fatty acids** are lipids that contain long-chain hydrocarbons terminated with a carboxylic acid functional group. Because the long hydrocarbon chain, fatty acids are **hydrophobic** (“water fearing”) or nonpolar. Fatty acids with hydrocarbon chains that contain only single bonds are called **saturated fatty acids** because they have the greatest number of hydrogen

atoms possible and are, therefore, “saturated” with hydrogen. Fatty acids with hydrocarbon chains containing at least one double bond are called **unsaturated fatty acids** because they have fewer hydrogen atoms. Saturated fatty acids have a straight, flexible carbon backbone, whereas unsaturated fatty acids have “kinks” in their carbon skeleton because each double bond causes a rigid bend of the carbon skeleton. These differences in saturated versus unsaturated fatty acid structure result in different properties for the corresponding lipids in which the fatty acids are incorporated. For example, lipids containing saturated fatty acids are solids at room temperature, whereas lipids containing unsaturated fatty acids are liquids.

A **triacylglycerol**, or **triglyceride**, is formed when three fatty acids are chemically linked to a glycerol molecule ([\[link\]](#)). Triglycerides are the primary components of adipose tissue (body fat), and are major constituents of sebum (skin oils). They play an important metabolic role, serving as efficient energy-storage molecules that can provide more than double the caloric content of both carbohydrates and proteins.

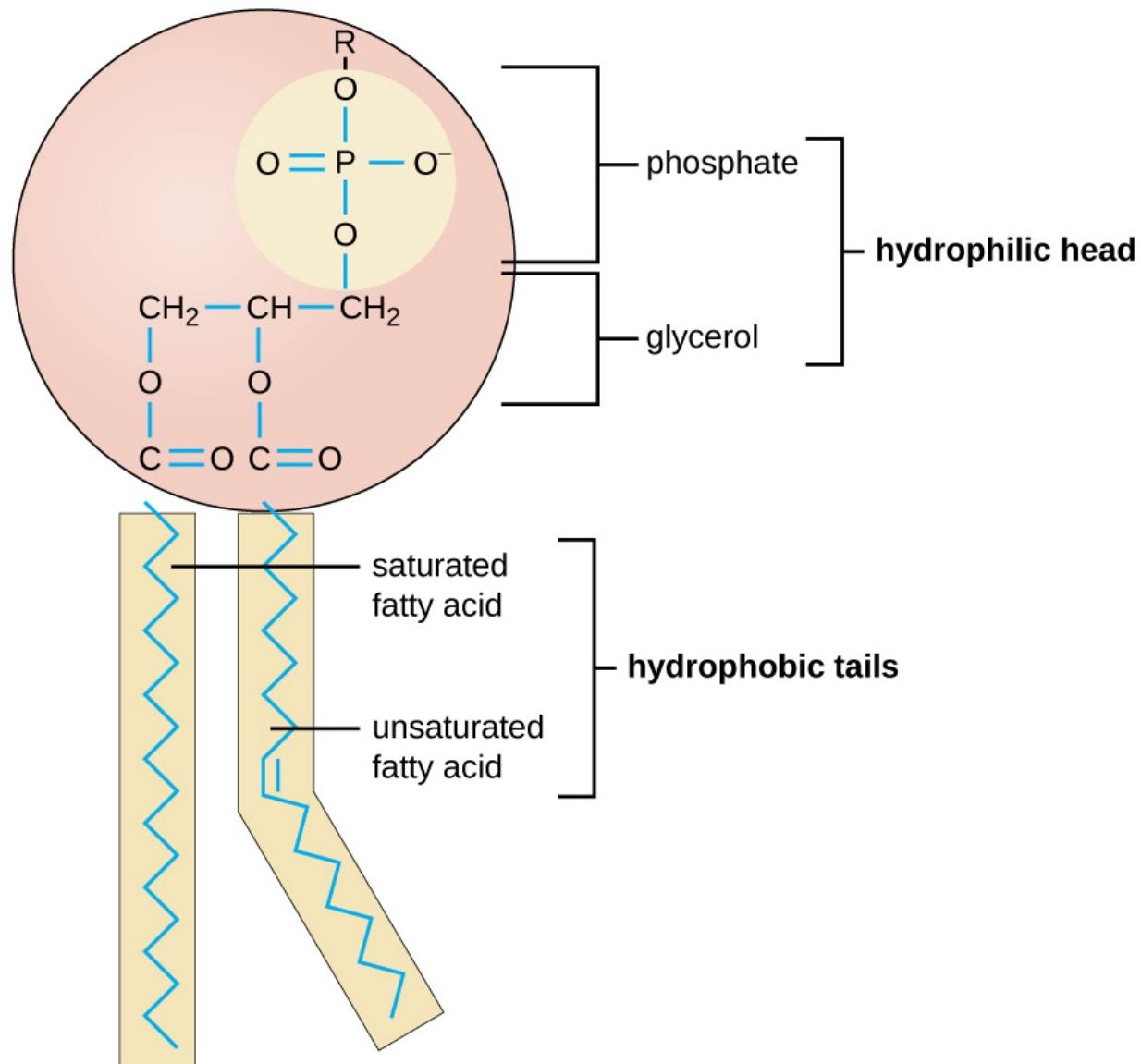
Three fatty acid chains are bound to glycerol by dehydration synthesis.



Triglycerides are composed of a glycerol molecule attached to three fatty acids by a dehydration synthesis reaction.

Phospholipids and Biological Membranes

Triglycerides are classified as simple lipids because they are formed from just two types of compounds: glycerol and fatty acids. In contrast, complex lipids contain at least one additional component, for example, a phosphate group (**phospholipids**) or a carbohydrate moiety (**glycolipids**). [\[link\]](#) depicts a typical phospholipid composed of two fatty acids linked to glycerol (a diglyceride). The two fatty acid carbon chains may be both saturated, both unsaturated, or one of each. Instead of another fatty acid molecule (as for triglycerides), the third binding position on the glycerol molecule is occupied by a modified phosphate group.

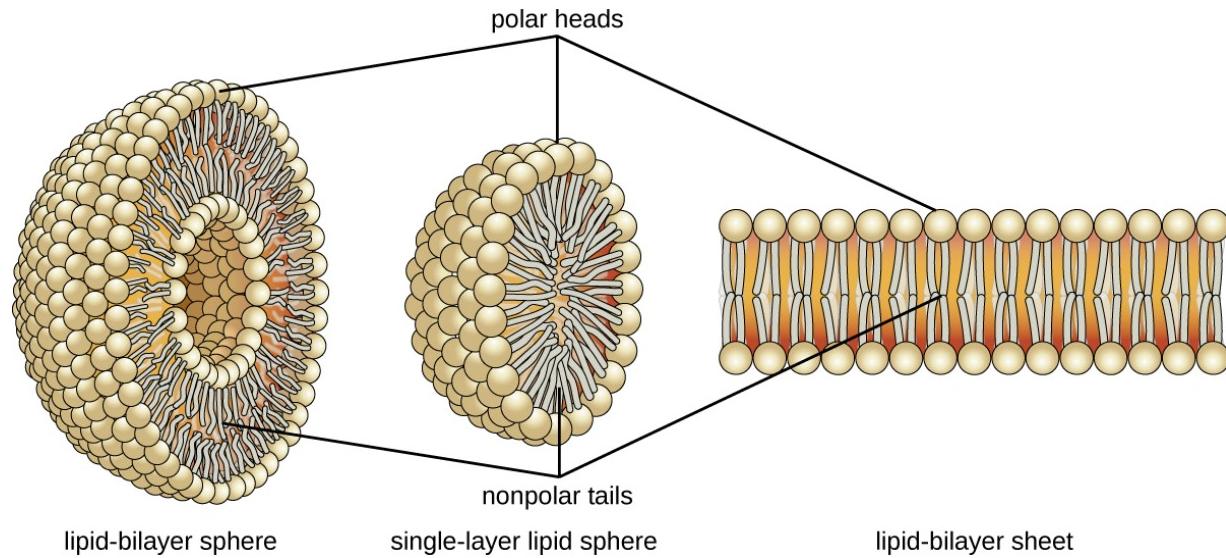


This illustration shows a phospholipid with two different fatty acids, one saturated and one unsaturated, bonded to the glycerol molecule. The unsaturated fatty acid has a slight kink in its structure due to the double bond.

The molecular structure of lipids results in unique behavior in aqueous environments. [\[link\]](#) depicts the structure of a triglyceride. Because all three substituents on the glycerol backbone are long hydrocarbon chains, these compounds are nonpolar and not significantly attracted to polar water molecules—they are hydrophobic. Conversely, phospholipids such as the one shown in [\[link\]](#) have a negatively charged phosphate group. Because the phosphate is charged, it is capable of strong attraction to water molecules and thus is **hydrophilic**, or “water loving.” The hydrophilic portion of the phospholipid is often referred to as a polar “head,” and the long hydrocarbon chains as nonpolar “tails.” A molecule presenting a hydrophobic portion and a hydrophilic moiety is said to be **amphipathic**. Notice the “R” designation within the hydrophilic head depicted in [\[link\]](#), indicating that a polar head group can be more complex than a simple phosphate moiety. Glycolipids are examples in which carbohydrates are bonded to the lipids’ head groups.

The amphipathic nature of phospholipids enables them to form uniquely functional structures in aqueous environments. As mentioned, the polar heads of these molecules are strongly attracted to water molecules, and the nonpolar tails are not. Because of their considerable lengths, these tails are, in fact, strongly attracted to one another. As a result, energetically stable, large-scale assemblies of phospholipid molecules are formed in which the hydrophobic tails congregate within enclosed regions, shielded from contact with water by the polar heads ([\[link\]](#)). The simplest of these structures are **micelles**, spherical assemblies containing a hydrophobic interior of phospholipid tails and an outer surface of polar head groups. Larger and more complex structures are created from **lipid-bilayer** sheets, or **unit membranes**, which are large, two-dimensional assemblies of phospholipids congregated tail to tail. The cell membranes of nearly all organisms are made from lipid-bilayer sheets, as are the membranes of many intracellular

components. These sheets may also form lipid-bilayer spheres that are the structural basis of vesicles and liposomes, subcellular components that play a role in numerous physiological functions.



Phospholipids tend to arrange themselves in aqueous solution forming liposomes, micelles, or lipid bilayer sheets. (credit: modification of work by Mariana Ruiz Villarreal)

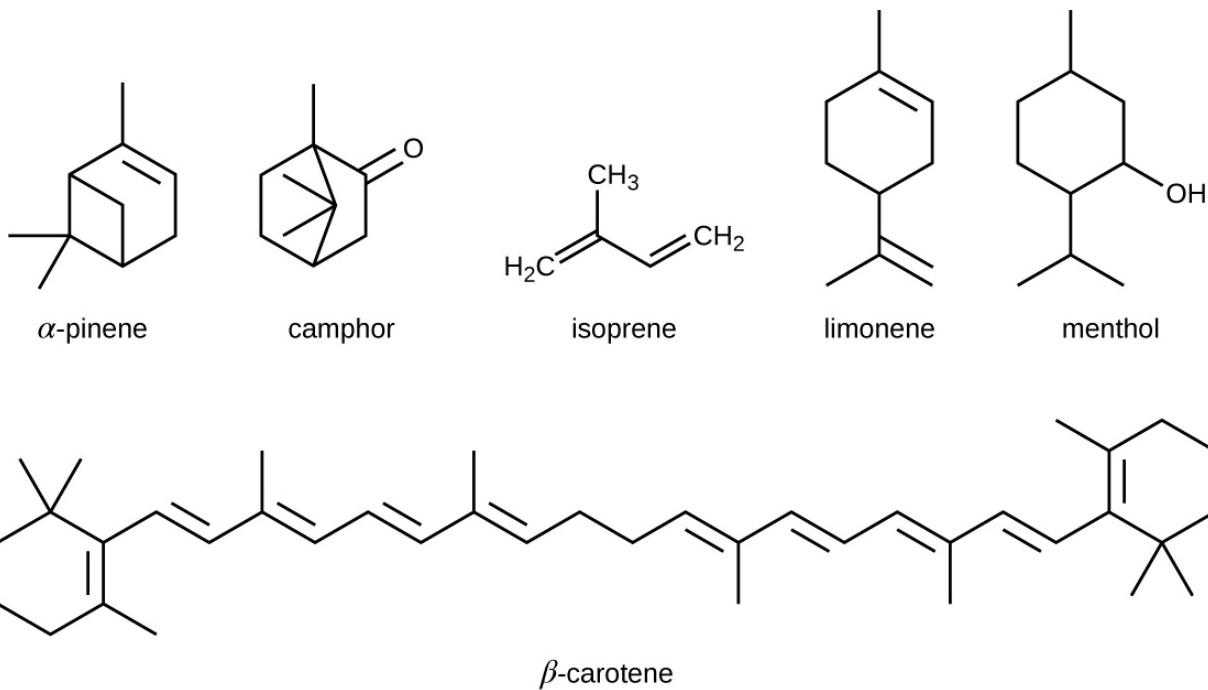
Note:

- How is the amphipathic nature of phospholipids significant?

Isoprenoids and Sterols

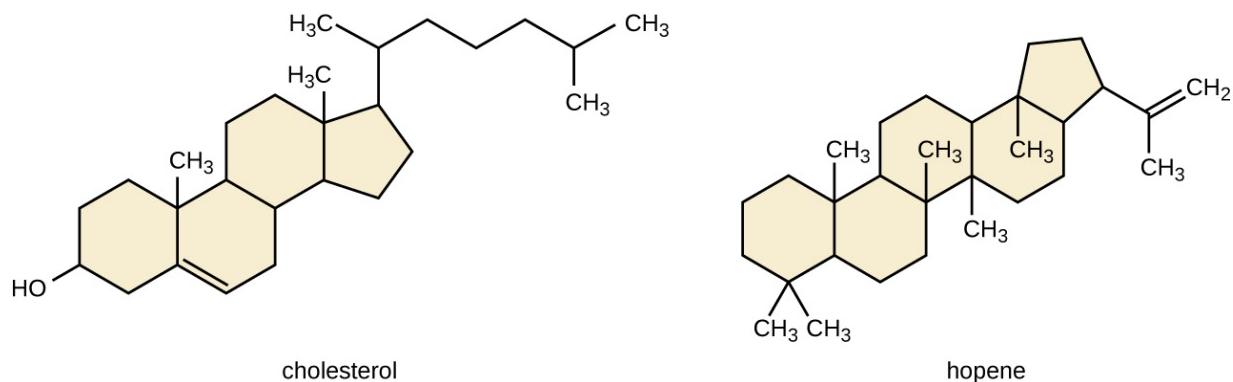
The **isoprenoids** are branched lipids, also referred to as terpenoids, that are formed by chemical modifications of the isoprene molecule ([\[link\]](#)). These

lipids play a wide variety of physiological roles in plants and animals, with many technological uses as pharmaceuticals (capsaicin), pigments (e.g., orange beta carotene, xanthophylls), and fragrances (e.g., menthol, camphor, limonene [lemon fragrance], and pinene [pine fragrance]). Long-chain isoprenoids are also found in hydrophobic oils and waxes. Waxes are typically water resistant and hard at room temperature, but they soften when heated and liquefy if warmed adequately. In humans, the main wax production occurs within the sebaceous glands of hair follicles in the skin, resulting in a secreted material called sebum, which consists mainly of triacylglycerol, wax esters, and the hydrocarbon squalene. There are many bacteria in the microbiota on the skin that feed on these lipids. One of the most prominent bacteria that feed on lipids is *Propionibacterium acnes*, which uses the skin's lipids to generate short-chain fatty acids and is involved in the production of acne.



Five-carbon isoprene molecules are chemically modified in various ways to yield isoprenoids.

Another type of lipids are **steroids**, complex, ringed structures that are found in cell membranes; some function as hormones. The most common types of steroids are **sterols**, which are steroids containing an OH group. These are mainly hydrophobic molecules, but also have hydrophilic hydroxyl groups. The most common sterol found in animal tissues is cholesterol. Its structure consists of four rings with a double bond in one of the rings, and a hydroxyl group at the sterol-defining position. The function of cholesterol is to strengthen cell membranes in eukaryotes and in bacteria without cell walls, such as *Mycoplasma*. Prokaryotes generally do not produce cholesterol, although bacteria produce similar compounds called hopanoids, which are also multiringed structures that strengthen bacterial membranes ([\[link\]](#)). Fungi and some protozoa produce a similar compound called ergosterol, which strengthens the cell membranes of these organisms.



Cholesterol and hopene (a hopanoid compound) are molecules that reinforce the structure of the cell membranes in eukaryotes and prokaryotes, respectively.

Note:
Liposomes



This [video](#) provides additional information about phospholipids and liposomes.

Key Concepts and Summary

- **Lipids** are composed mainly of carbon and hydrogen, but they can also contain oxygen, nitrogen, sulfur, and phosphorous. They provide nutrients for organisms, store carbon and energy, play structural roles in membranes, and function as hormones, pharmaceuticals, fragrances, and pigments.
- Fatty acids are long-chain hydrocarbons with a carboxylic acid functional group. Their relatively long nonpolar hydrocarbon chains make them **hydrophobic**. Fatty acids with no double bonds are **saturated**; those with double bonds are **unsaturated**.
- Fatty acids chemically bond to glycerol to form structurally essential lipids such as **triglycerides** and **phospholipids**. Triglycerides comprise three fatty acids bonded to glycerol, yielding a hydrophobic molecule. Phospholipids contain both hydrophobic hydrocarbon chains and polar head groups, making them **amphiphatic** and capable of forming uniquely functional large scale structures.
- Biological membranes are large-scale structures based on phospholipid bilayers that provide hydrophilic exterior and interior surfaces suitable for aqueous environments, separated by an intervening hydrophobic layer. These bilayers are the structural basis for cell membranes in most organisms, as well as subcellular components such as vesicles.
- **Isoprenoids** are lipids derived from isoprene molecules that have many physiological roles and a variety of commercial applications.

- A wax is a long-chain isoprenoid that is typically water resistant; an example of a wax-containing substance is sebum, produced by sebaceous glands in the skin. **Steroids** are lipids with complex, ringed structures that function as structural components of cell membranes and as hormones. **Sterols** are a subclass of steroids containing a hydroxyl group at a specific location on one of the molecule's rings; one example is cholesterol.
- Bacteria produce hopanoids, structurally similar to cholesterol, to strengthen bacterial membranes. Fungi and protozoa produce a strengthening agent called ergosterol.

Critical Thinking

Exercise:

Problem:

Microorganisms can thrive under many different conditions, including high-temperature environments such as hot springs. To function properly, cell membranes have to be in a fluid state. How do you expect the fatty acid content (saturated versus unsaturated) of bacteria living in high-temperature environments might compare with that of bacteria living in more moderate temperatures?

Proteins

LEARNING OBJECTIVES

- Describe the fundamental structure of an amino acid
- Describe the chemical structures of proteins
- Summarize the unique characteristics of proteins

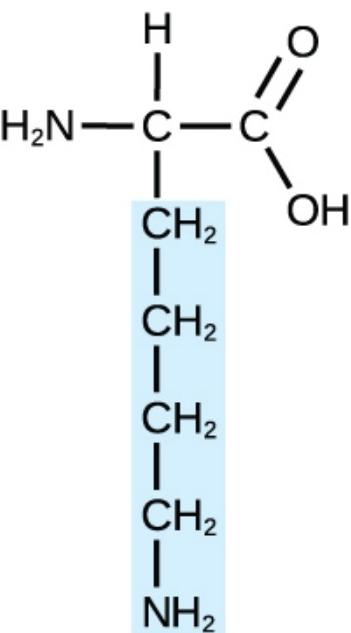
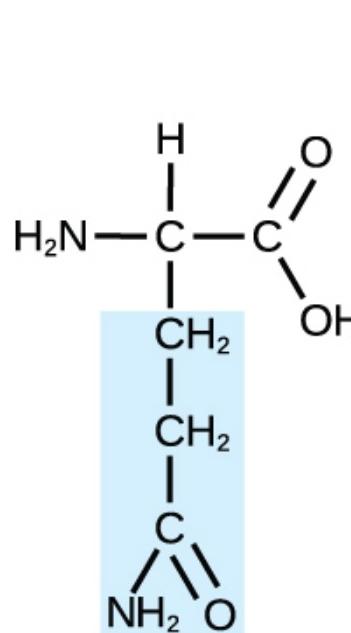
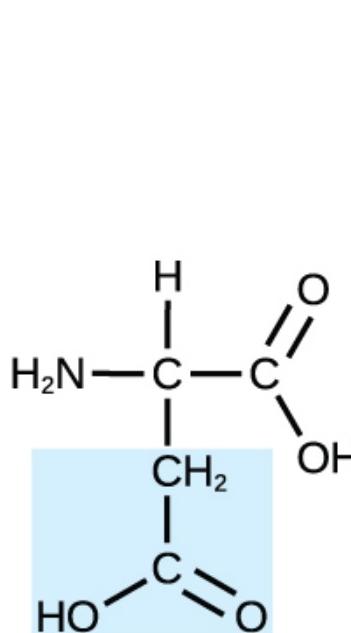
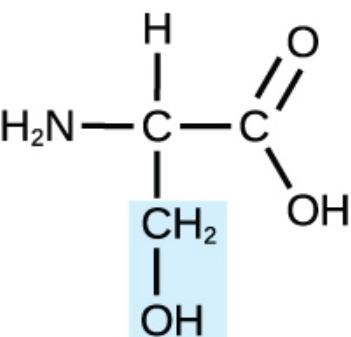
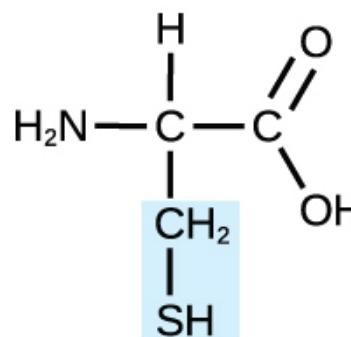
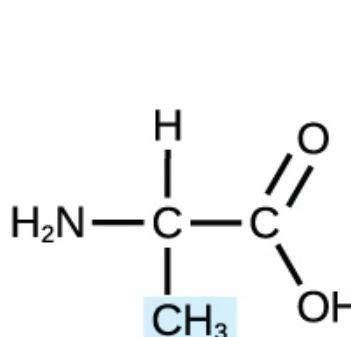
At the beginning of this chapter, a famous experiment was described in which scientists synthesized amino acids under conditions simulating those present on earth long before the evolution of life as we know it. These compounds are capable of bonding together in essentially any number, yielding molecules of essentially any size that possess a wide array of physical and chemical properties and perform numerous functions vital to all organisms. The molecules derived from amino acids can function as structural components of cells and subcellular entities, as sources of nutrients, as atom- and energy-storage reservoirs, and as functional species such as hormones, enzymes, receptors, and transport molecules.

Amino Acids and Peptide Bonds

An **amino acid** is an organic molecule in which a hydrogen atom, a carboxyl group ($-COOH$), and an amino group ($-NH_2$) are all bonded to the same carbon atom, the so-called α carbon. The fourth group bonded to the α carbon varies among the different amino acids and is called a residue or a **side chain**, represented in structural formulas by the letter R . A residue is a monomer that results when two or more amino acids combine and remove water molecules. The primary structure of a protein, a peptide chain, is made of amino acid residues. The unique characteristics of the functional

groups and *R* groups allow these components of the amino acids to form hydrogen, ionic, and disulfide bonds, along with polar/nonpolar interactions needed to form secondary, tertiary, and quaternary protein structures. These groups are composed primarily of carbon, hydrogen, oxygen, nitrogen, and sulfur, in the form of hydrocarbons, acids, amides, alcohols, and amines. A few examples illustrating these possibilities are provided in [[link](#)].

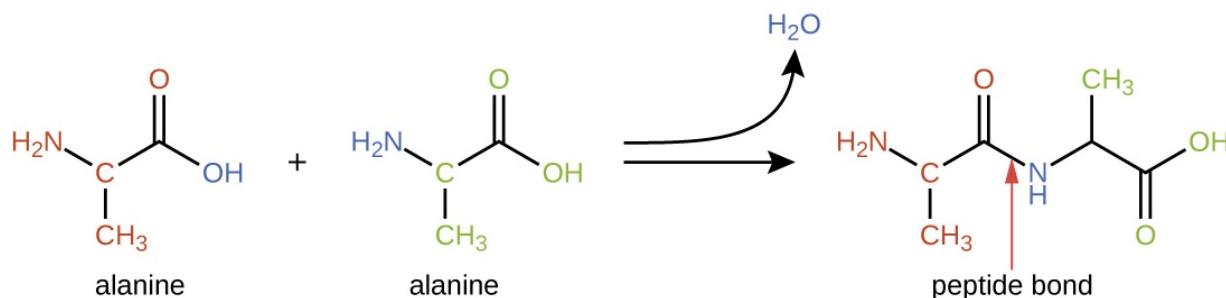
Some Amino Acids and Their Structures

 <p>lysine</p>	 <p>glutamine</p>	 <p>aspartate</p>
 <p>serine</p>	 <p>cysteine</p>	 <p>alanine</p>

*Blue shading indicates R group.

Amino acids may chemically bond together by reaction of the carboxylic acid group of one molecule with the amine group of another. This reaction forms a **peptide bond** and a water molecule and is another example of dehydration synthesis ([\[link\]](#)). Molecules formed by chemically linking relatively modest numbers of amino acids (approximately 50 or fewer) are called peptides, and prefixes are often used to specify these numbers:

dipeptides (two amino acids), tripeptides (three amino acids), and so forth. More generally, the approximate number of amino acids is designated: **oligopeptides** are formed by joining up to approximately 20 amino acids, whereas **polypeptides** are synthesized from up to approximately 50 amino acids. When the number of amino acids linked together becomes very large, or when multiple polypeptides are used as building subunits, the macromolecules that result are called **proteins**. The continuously variable length (the number of monomers) of these biopolymers, along with the variety of possible *R* groups on each amino acid, allows for a nearly unlimited diversity in the types of proteins that may be formed.



Peptide bond formation is a dehydration synthesis reaction. The carboxyl group of the first amino acid (alanine) is linked to the amino group of the incoming second amino acid (alanine). In the process, a molecule of water is released.

Note:

- How many amino acids are in polypeptides?

Protein Structure

The size (length) and specific amino acid sequence of a protein are major determinants of its shape, and the shape of a protein is critical to its function. For example, in the process of biological nitrogen fixation (see [Biogeochemical Cycles](#)), soil microorganisms collectively known as rhizobia symbiotically interact with roots of legume plants such as soybeans, peanuts, or beans to form a novel structure called a nodule on the plant roots. The plant then produces a carrier protein called leghemoglobin, a protein that carries nitrogen or oxygen. Leghemoglobin binds with a very high affinity to its substrate oxygen at a specific region of the protein where the shape and amino acid sequence are appropriate (the active site). If the shape or chemical environment of the active site is altered, even slightly, the substrate may not be able to bind as strongly, or it may not bind at all. Thus, for the protein to be fully active, it must have the appropriate shape for its function.

Protein structure is categorized in terms of four levels: primary, secondary, tertiary, and quaternary. The **primary structure** is simply the sequence of amino acids that make up the polypeptide chain. [\[link\]](#) depicts the primary structure of a protein.

The chain of amino acids that defines a protein's primary structure is not rigid, but instead is flexible because of the nature of the bonds that hold the amino acids together. When the chain is sufficiently long, hydrogen bonding may occur between amine and carbonyl functional groups within the peptide backbone (excluding the *R* side group), resulting in localized folding of the polypeptide chain into helices and sheets. These shapes constitute a protein's **secondary structure**. The most common secondary structures are the α -helix and β -pleated sheet. In the **α -helix** structure, the helix is held by hydrogen bonds between the oxygen atom in a carbonyl group of one amino acid and the hydrogen atom of the amino group that is just four amino acid units farther along the chain. In the **β -pleated sheet**, the pleats are formed by similar hydrogen bonds between continuous sequences of carbonyl and amino groups that are further separated on the backbone of the polypeptide chain ([\[link\]](#)).

The next level of protein organization is the **tertiary structure**, which is the large-scale three-dimensional shape of a single polypeptide chain.

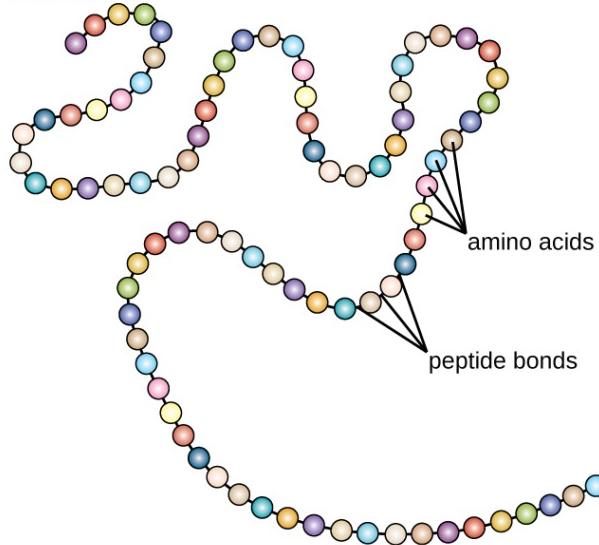
Tertiary structure is determined by interactions between amino acid residues that are far apart in the chain. A variety of interactions give rise to protein tertiary structure, such as **disulfide bridges**, which are bonds between the sulfhydryl ($-SH$) functional groups on amino acid side groups; hydrogen bonds; ionic bonds; and hydrophobic interactions between nonpolar side chains. All these interactions, weak and strong, combine to determine the final three-dimensional shape of the protein and its function ([\[link\]](#)).

The process by which a polypeptide chain assumes a large-scale, three-dimensional shape is called protein folding. Folded proteins that are fully functional in their normal biological role are said to possess a **native structure**. When a protein loses its three-dimensional shape, it may no longer be functional. These unfolded proteins are **denatured**. Denaturation implies the loss of the secondary structure and tertiary structure (and, if present, the quaternary structure) without the loss of the primary structure.

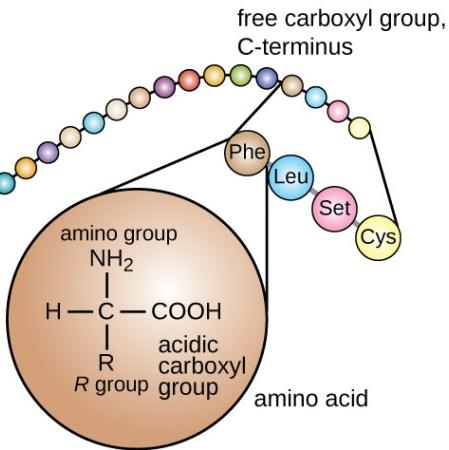
Some proteins are assemblies of several separate polypeptides, also known as protein subunits. These proteins function adequately only when all subunits are present and appropriately configured. The interactions that hold these subunits together constitute the **quaternary structure** of the protein. The overall quaternary structure is stabilized by relatively weak interactions. Hemoglobin, for example, has a quaternary structure of four globular protein subunits: two α and two β polypeptides, each one containing an iron-based heme ([\[link\]](#)).

Another important class of proteins is the **conjugated proteins** that have a nonprotein portion. If the conjugated protein has a carbohydrate attached, it is called a **glycoprotein**. If it has a lipid attached, it is called a **lipoprotein**. These proteins are important components of membranes. [\[link\]](#) summarizes the four levels of protein structure.

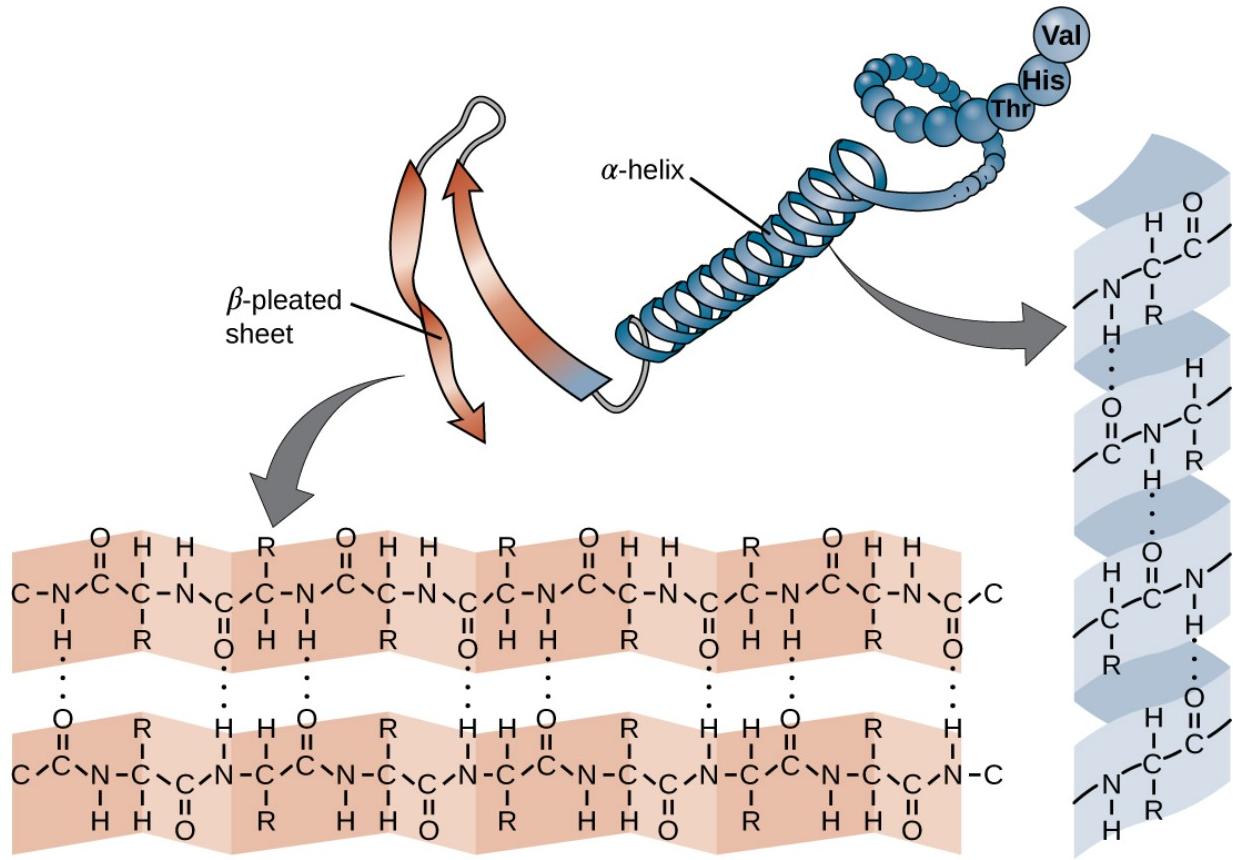
free amino group,
N-terminus



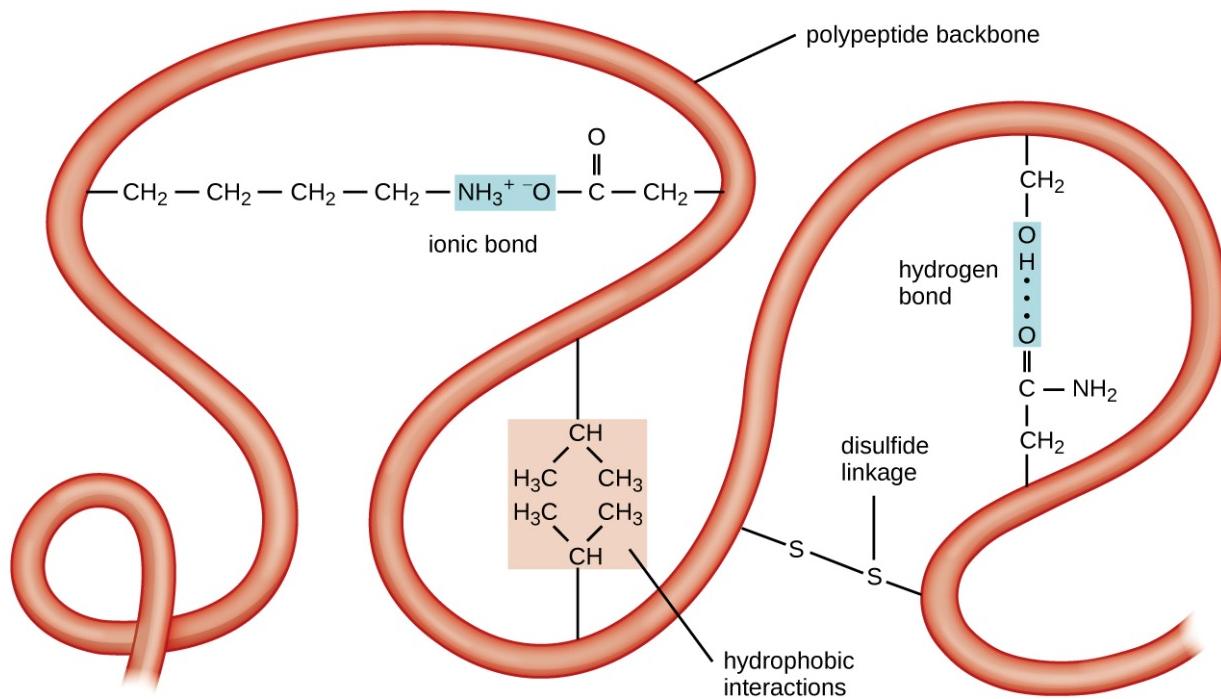
The primary protein structure
is the chain of amino acids
that makes up the protein.



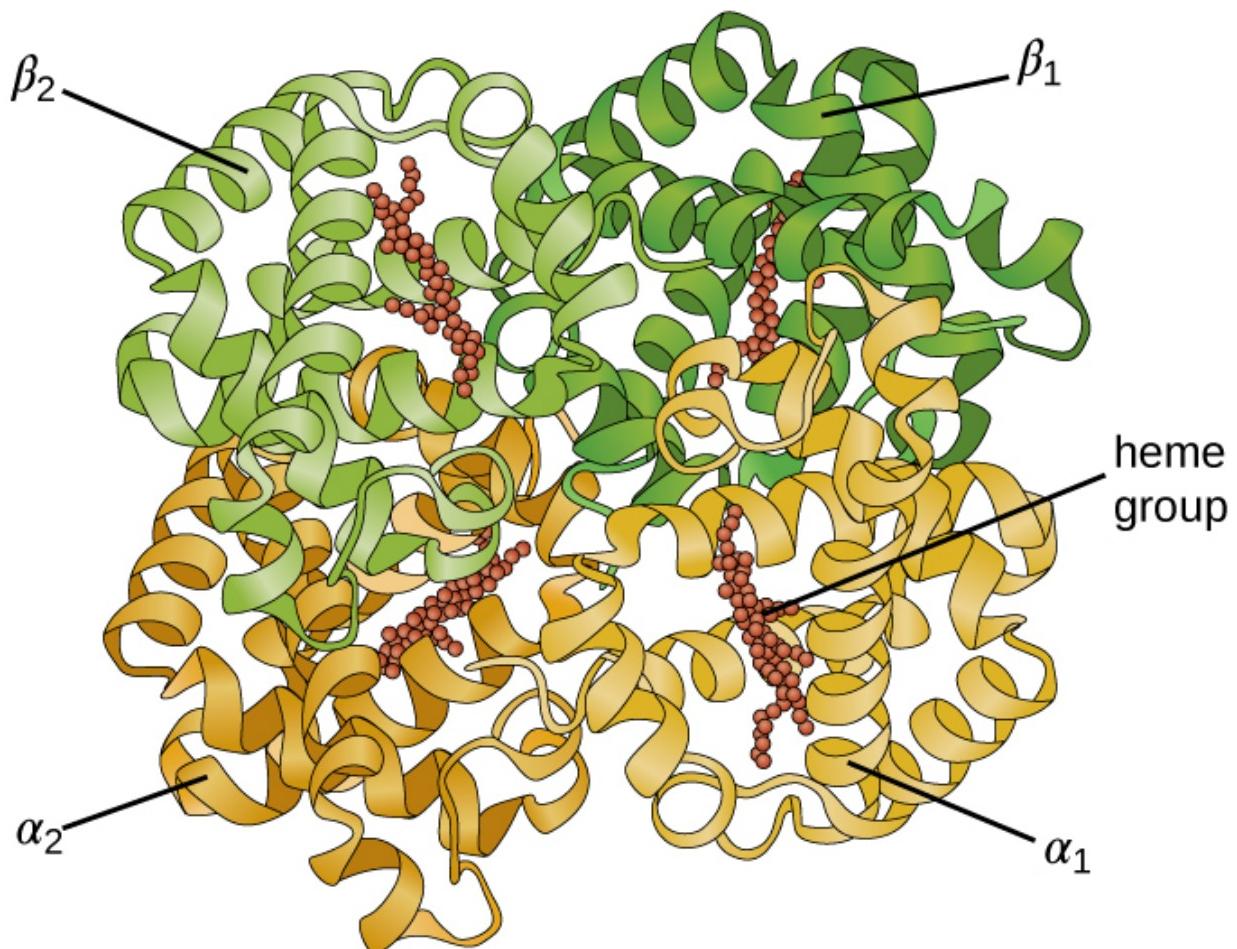
The primary structure of a protein is the sequence of amino acids.
(credit: modification of work by National Human Genome Research
Institute)



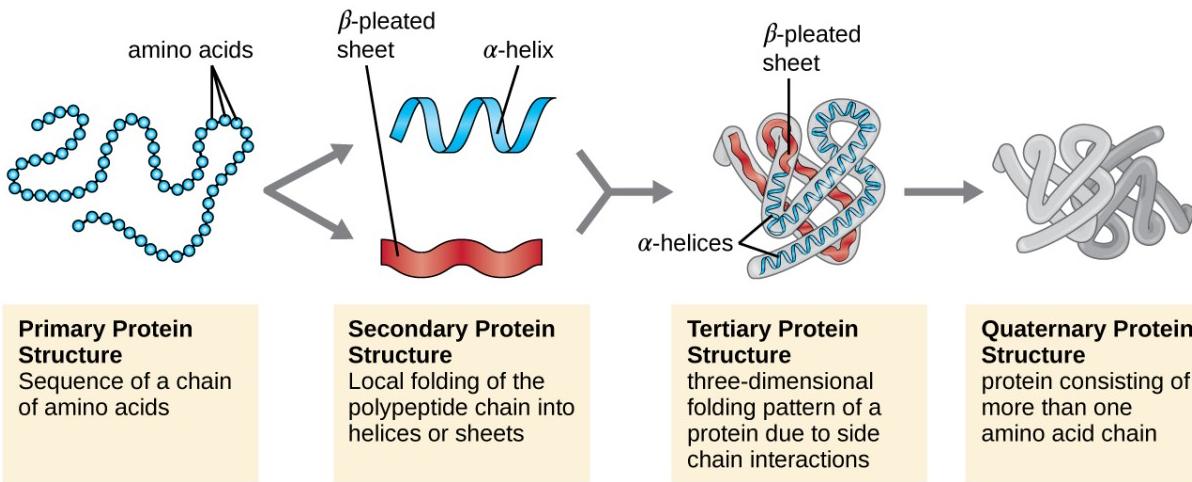
The secondary structure of a protein may be an α -helix or a β -pleated sheet, or both.



The tertiary structure of proteins is determined by a variety of attractive forces, including hydrophobic interactions, ionic bonding, hydrogen bonding, and disulfide linkages.



A hemoglobin molecule has two α and two β polypeptides together with four heme groups.



Protein structure has four levels of organization. (credit: modification of work by National Human Genome Research Institute)

Note:

- What can happen if a protein's primary, secondary, tertiary, or quaternary structure is changed?

Note:

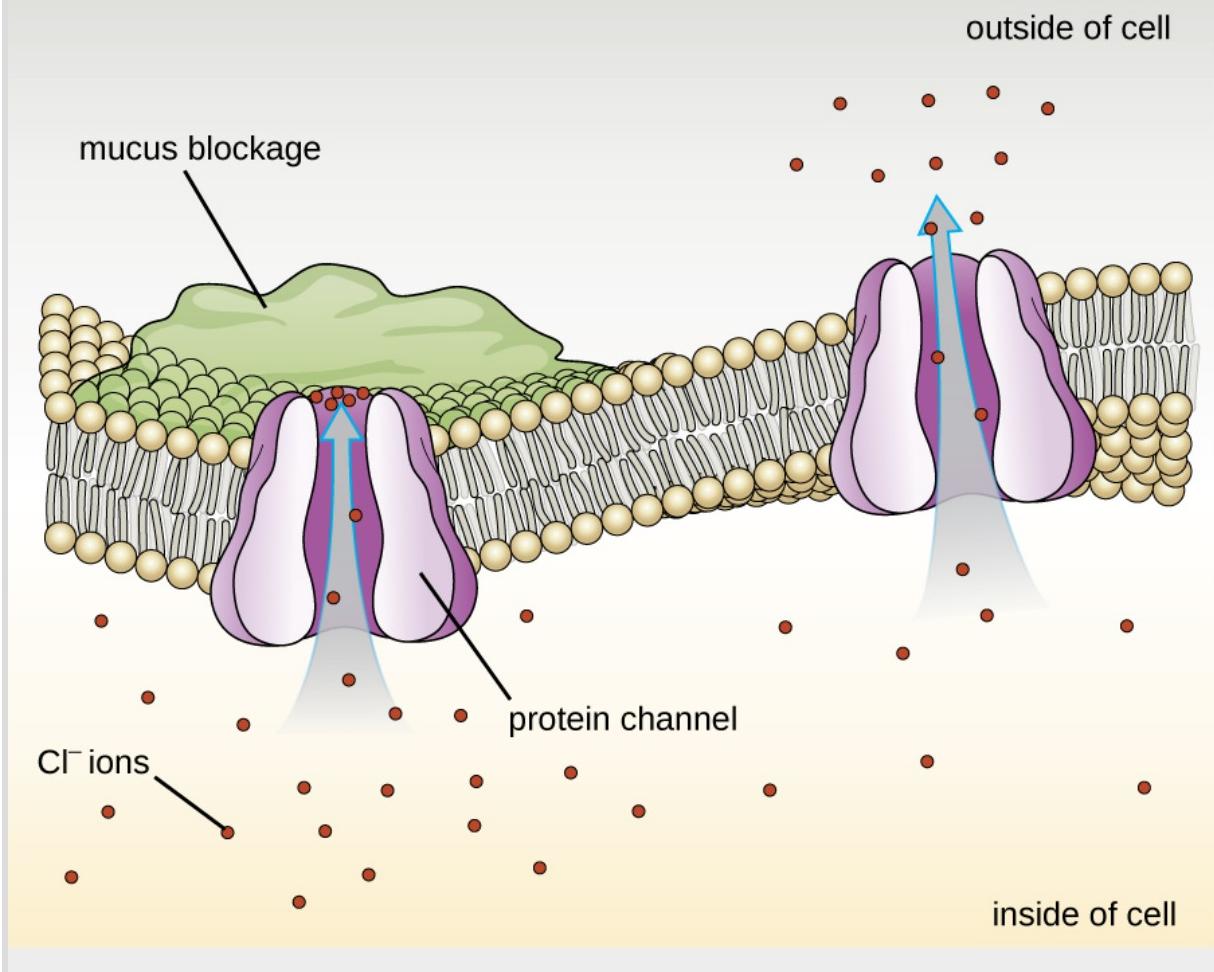
Primary Structure, Dysfunctional Proteins, and Cystic Fibrosis

Proteins associated with biological membranes are classified as extrinsic or intrinsic. Extrinsic proteins, also called peripheral proteins, are loosely associated with one side of the membrane. Intrinsic proteins, or integral proteins, are embedded in the membrane and often function as part of transport systems as transmembrane proteins. Cystic fibrosis (CF) is a human genetic disorder caused by a change in the transmembrane protein. It affects mostly the lungs but may also affect the pancreas, liver, kidneys, and intestine. CF is caused by a loss of the amino acid phenylalanine in a cystic fibrosis transmembrane protein (CFTR). The loss of one amino acid

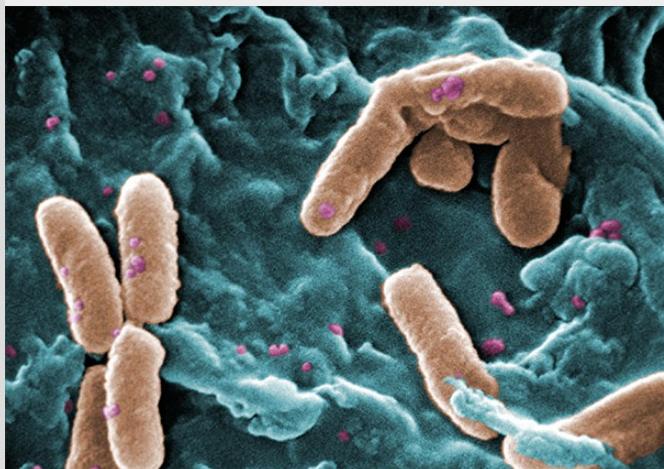
changes the primary structure of a protein that normally helps transport salt and water in and out of cells ([\[link\]](#)).

The change in the primary structure prevents the protein from functioning properly, which causes the body to produce unusually thick mucus that clogs the lungs and leads to the accumulation of sticky mucus. The mucus obstructs the pancreas and stops natural enzymes from helping the body break down food and absorb vital nutrients.

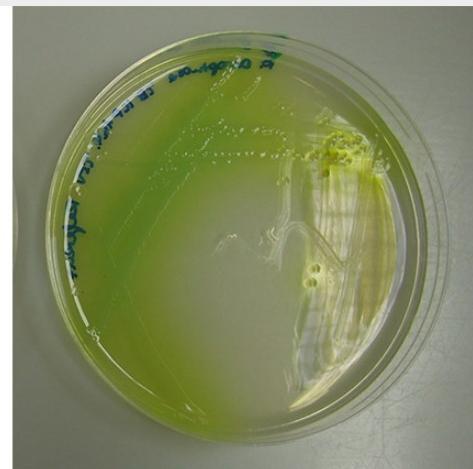
In the lungs of individuals with cystic fibrosis, the altered mucus provides an environment where bacteria can thrive. This colonization leads to the formation of biofilms in the small airways of the lungs. The most common pathogens found in the lungs of patients with cystic fibrosis are *Pseudomonas aeruginosa* ([\[link\]](#)) and *Burkholderia cepacia*. *Pseudomonas* differentiates within the biofilm in the lung and forms large colonies, called “mucoid” *Pseudomonas*. The colonies have a unique pigmentation that shows up in laboratory tests ([\[link\]](#)) and provides physicians with the first clue that the patient has CF (such colonies are rare in healthy individuals).



The normal CFTR protein is a channel protein that helps salt (sodium chloride) move in and out of cells.



(a)



(b)

(a) A scanning electron micrograph shows the opportunistic bacterium *Pseudomonas aeruginosa*. (b) Pigment-producing *P. aeruginosa* on cetrimide agar shows the green pigment called pyocyanin. (credit a: modification of work by the Centers for Disease Control and Prevention)

Note:



For more information about cystic fibrosis, visit the [Cystic Fibrosis Foundation](#) website.

Key Concepts and Summary

- Amino acids are small molecules essential to all life. Each has an α carbon to which a hydrogen atom, carboxyl group, and amine group are bonded. The fourth bonded group, represented by R , varies in chemical composition, size, polarity, and charge among different amino acids, providing variation in properties.
- **Peptides** are polymers formed by the linkage of amino acids via dehydration synthesis. The bonds between the linked amino acids are called **peptide bonds**. The number of amino acids linked together may vary from a few to many.
- **Proteins** are polymers formed by the linkage of a very large number of amino acids. They perform many important functions in a cell, serving as nutrients and enzymes; storage molecules for carbon, nitrogen, and energy; and structural components.
- The structure of a protein is a critical determinant of its function and is described by a graduated classification: **primary, secondary, tertiary, and quaternary**. The **native structure** of a protein may be disrupted by **denaturation**, resulting in loss of its higher-order structure and its biological function.
- Some proteins are formed by several separate protein subunits, the interaction of these subunits composing the **quaternary structure** of the protein complex.
- **Conjugated proteins** have a nonpolypeptide portion that can be a carbohydrate (forming a **glycoprotein**) or a lipid fraction (forming a **lipoprotein**). These proteins are important components of membranes.

Critical Thinking

Exercise:

Problem:

Heating a protein sufficiently may cause it to denature. Considering the definition of denaturation, what does this statement say about the strengths of peptide bonds in comparison to hydrogen bonds?

Using Biochemistry to Identify Microorganisms

LEARNING OBJECTIVES

- Describe examples of biosynthesis products within a cell that can be detected to identify bacteria
- Discuss additional information a biochemical characterization of an organism can provide

Accurate identification of bacterial isolates is essential in a clinical microbiology laboratory because the results often inform decisions about treatment that directly affect patient outcomes. For example, cases of food poisoning require accurate identification of the causative agent so that physicians can prescribe appropriate treatment. Likewise, it is important to accurately identify the causative pathogen during an outbreak of disease so that appropriate strategies can be employed to contain the epidemic.

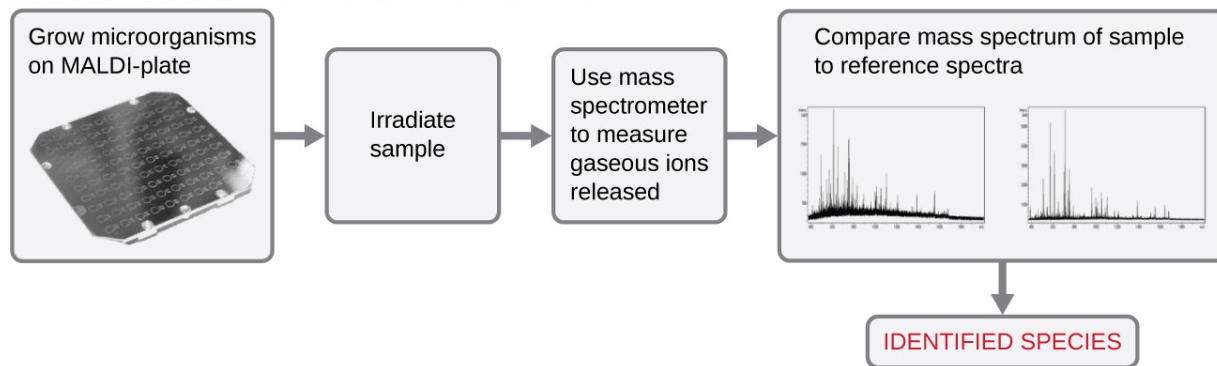
There are many ways to detect, characterize, and identify microorganisms. Some methods rely on phenotypic biochemical characteristics, while others use genotypic identification. The biochemical characteristics of a bacterium provide many traits that are useful for classification and identification. Analyzing the nutritional and metabolic capabilities of the bacterial isolate is a common approach for determining the genus and the species of the bacterium. Some of the most important metabolic pathways that bacteria use to survive will be discussed in [Microbial Metabolism](#). In this section, we will discuss a few methods that use biochemical characteristics to identify microorganisms.

Some microorganisms store certain compounds as granules within their cytoplasm, and the contents of these granules can be used for identification purposes. For example, poly- β -hydroxybutyrate (PHB) is a carbon- and energy-storage compound found in some nonfluorescent bacteria of the genus *Pseudomonas*. Different species within this genus can be classified by the presence or the absence of PHB and fluorescent pigments. The human pathogen *P. aeruginosa* and the plant pathogen *P. syringae* are two examples of fluorescent *Pseudomonas* species that do not accumulate PHB granules.

Other systems rely on biochemical characteristics to identify microorganisms by their biochemical reactions, such as carbon utilization and other metabolic tests. In small laboratory settings or in teaching laboratories, those assays are carried out using a limited number of test tubes. However, more modern systems, such as the one developed by Biolog, Inc., are based on panels of biochemical reactions performed simultaneously and analyzed by software. Biolog's system identifies cells based on their ability to metabolize certain biochemicals and on their physiological properties, including pH and chemical sensitivity. It uses all major classes of biochemicals in its analysis. Identifications can be performed manually or with the semi- or fully automated instruments.

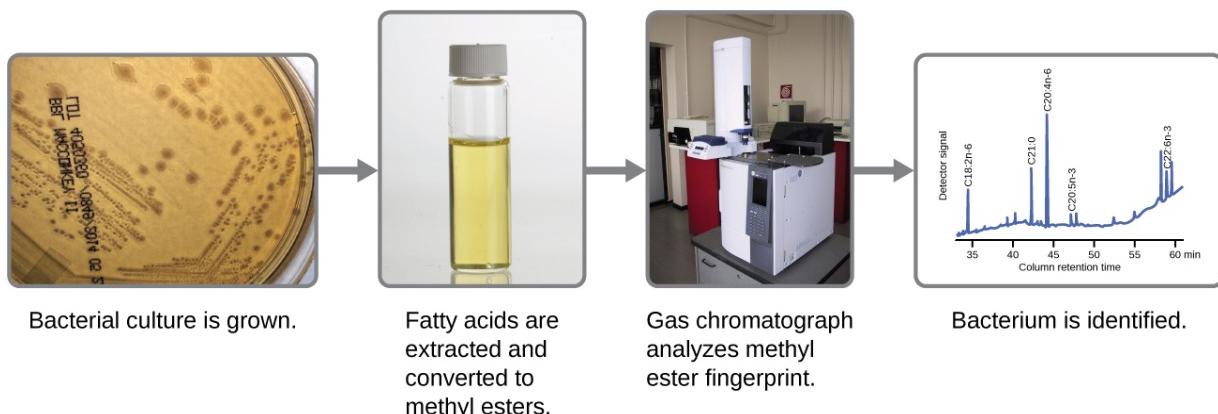
Another automated system identifies microorganisms by determining the specimen's mass spectrum and then comparing it to a database that contains known mass spectra for thousands of microorganisms. This method is based on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) and uses disposable MALDI plates on which the microorganism is mixed with a specialized matrix reagent ([\[link\]](#)). The sample/reagent mixture is irradiated with a high-intensity pulsed ultraviolet laser, resulting in the ejection of gaseous ions generated from the various chemical constituents of the microorganism. These gaseous ions are collected and accelerated through the mass spectrometer, with ions traveling at a velocity determined by their mass-to-charge ratio (m/z), thus, reaching the detector at different times. A plot of detector signal versus m/z yields a mass spectrum for the organism that is uniquely related to its biochemical composition. Comparison of the mass spectrum to a library of reference

spectra obtained from identical analyses of known microorganisms permits identification of the unknown microbe.



MALDI-TOF methods are now routinely used for diagnostic procedures in clinical microbiology laboratories. This technology is able to rapidly identify some microorganisms that cannot be readily identified by more traditional methods. (credit “MALDI plate photo”: modification of work by Chen Q, Liu T, Chen G; credit “graphs”: modification of work by Bailes J, Vidal L, Ivanov DA, Soloviev M)

Microbes can also be identified by measuring their unique lipid profiles. As we have learned, fatty acids of lipids can vary in chain length, presence or absence of double bonds, and number of double bonds, hydroxyl groups, branches, and rings. To identify a microbe by its lipid composition, the fatty acids present in their membranes are analyzed. A common biochemical analysis used for this purpose is a technique used in clinical, public health, and food laboratories. It relies on detecting unique differences in fatty acids and is called **fatty acid methyl ester (FAME) analysis**. In a FAME analysis, fatty acids are extracted from the membranes of microorganisms, chemically altered to form volatile methyl esters, and analyzed by gas chromatography (GC). The resulting GC chromatogram is compared with reference chromatograms in a database containing data for thousands of bacterial isolates to identify the unknown microorganism ([\[link\]](#)).



Fatty acid methyl ester (FAME) analysis in bacterial identification results in a chromatogram unique to each bacterium. Each peak in the gas chromatogram corresponds to a particular fatty acid methyl ester and its height is proportional to the amount present in the cell. (credit “culture”: modification of work by the Centers for Disease Control and Prevention; credit “graph”: modification of work by Zhang P. and Liu P.)

A related method for microorganism identification is called **phospholipid-derived fatty acids (PLFA) analysis**. Membranes are mostly composed of phospholipids, which can be saponified (hydrolyzed with alkali) to release the fatty acids. The resulting fatty acid mixture is then subjected to FAME analysis, and the measured lipid profiles can be compared with those of known microorganisms to identify the unknown microorganism.

Bacterial identification can also be based on the proteins produced under specific growth conditions within the human body. These types of identification procedures are called **proteomic analysis**. To perform proteomic analysis, proteins from the pathogen are first separated by high-pressure liquid chromatography (HPLC), and the collected fractions are then digested to yield smaller peptide fragments. These peptides are identified by mass spectrometry and compared with those of known microorganisms to identify the unknown microorganism in the original specimen.

Microorganisms can also be identified by the carbohydrates attached to proteins (glycoproteins) in the plasma membrane or cell wall. Antibodies and other carbohydrate-binding proteins can attach to specific carbohydrates on cell surfaces, causing the cells to clump together. Serological tests (e.g., the Lancefield groups tests, which are used for identification of *Streptococcus* species) are performed to detect the unique carbohydrates located on the surface of the cell.

Key Concepts and Summary

- Accurate identification of bacteria is essential in a clinical laboratory for diagnostic and management of epidemics, pandemics, and food poisoning caused by bacterial outbreaks.
- The phenotypic identification of microorganisms involves using observable traits, including profiles of structural components such as lipids, biosynthetic products such as sugars or amino acids, or storage compounds such as poly- β -hydroxybutyrate.
- An unknown microbe may be identified from the unique mass spectrum produced when it is analyzed by **matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF)**.
- Microbes can be identified by determining their lipid compositions, using **fatty acid methyl esters (FAME)** or **phospholipid-derived fatty acids (PLFA)** analysis.
- **Proteomic analysis**, the study of all accumulated proteins of an organism; can also be used for bacterial identification.
- Glycoproteins in the plasma membrane or cell wall structures can bind to lectins or antibodies and can be used for identification.

Short Answer

Exercise:

Problem:

Compare MALDI-TOF, FAME, and PLFA, and explain how each technique would be used to identify pathogens.

Introduction class="introduction"

Prokaryotes have great metabolic diversity with important consequences to other forms of life. Acidic mine drainage (left) is a serious environmental problem resulting from the introduction of water and oxygen to sulfide-oxidizing bacteria during mining processes.

These bacteria produce large amounts of sulfuric acid as a byproduct of their metabolism, resulting in a low-pH

environment
that can kill
many aquatic
plants and
animals. On
the other
hand, some
prokaryotes
are essential
to other life
forms. Root
nodules of
many plants
(right) house
nitrogen-
fixing
bacteria that
convert
atmospheric
nitrogen into
ammonia,
providing a
usable
nitrogen
source for
these plants.
(credit left:
modification
of work by D.
Hardesty,
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Celmow SR,
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Madsen LH,
and Guinel
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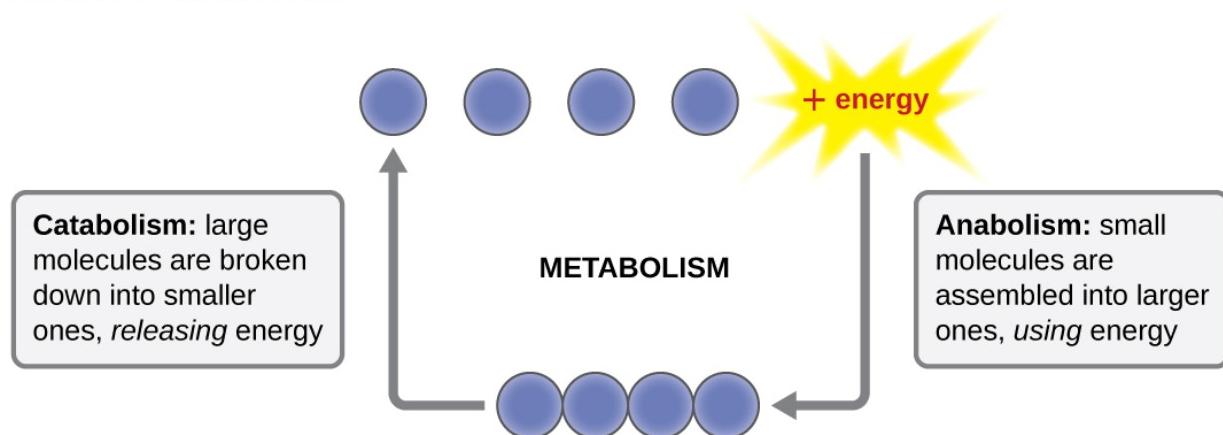
Throughout earth's history, microbial metabolism has been a driving force behind the development and maintenance of the planet's biosphere. Eukaryotic organisms such as plants and animals typically depend on organic molecules for energy, growth, and reproduction. Prokaryotes, on the other hand, can metabolize a wide range of organic as well as inorganic matter, from complex organic molecules like cellulose to inorganic molecules and ions such as atmospheric nitrogen (N_2), molecular hydrogen (H_2), sulfide (S^{2-}), manganese (II) ions (Mn^{2+}), ferrous iron (Fe^{2+}), and ferric iron (Fe^{3+}), to name a few. By metabolizing such substances, microbes chemically convert them to other forms. In some cases, microbial metabolism produces chemicals that can be harmful to other organisms; in others, it produces substances that are essential to the metabolism and survival of other life forms ([\[link\]](#)).

Energy, Matter, and Enzymes

LEARNING OBJECTIVES

- Define and describe metabolism
- Compare and contrast autotrophs and heterotrophs
- Describe the importance of oxidation-reduction reactions in metabolism
- Describe why ATP, FAD, NAD⁺, and NADP⁺ are important in a cell
- Identify the structure and structural components of an enzyme
- Describe the differences between competitive and noncompetitive enzyme inhibitors

The term used to describe all of the chemical reactions inside a cell is **metabolism** ([\[link\]](#)). Cellular processes such as the building or breaking down of complex molecules occur through series of stepwise, interconnected chemical reactions called metabolic pathways. Reactions that are spontaneous and release energy are **exergonic reactions**, whereas **endergonic reactions** require energy to proceed. The term **anabolism** refers to those endergonic metabolic pathways involved in biosynthesis, converting simple molecular building blocks into more complex molecules, and fueled by the use of cellular energy. Conversely, the term **catabolism** refers to exergonic pathways that break down complex molecules into simpler ones. Molecular energy stored in the bonds of complex molecules is released in catabolic pathways and harvested in such a way that it can be used to produce high-energy molecules, which are used to drive anabolic pathways. Thus, in terms of energy and molecules, cells are continually balancing catabolism with anabolism.



Metabolism includes catabolism and anabolism. Anabolic pathways require energy to

synthesize larger molecules. Catabolic pathways generate energy by breaking down larger molecules. Both types of pathways are required for maintaining the cell's energy balance.

Classification by Carbon and Energy Source

Organisms can be identified according to the source of carbon they use for metabolism as well as their energy source. The prefixes auto- ("self") and hetero- ("other") refer to the origins of the carbon sources various organisms can use. Organisms that convert inorganic carbon dioxide (CO_2) into organic carbon compounds are **autotrophs**. Plants and cyanobacteria are well-known examples of autotrophs. Conversely, **heterotrophs** rely on more complex organic carbon compounds as nutrients; these are provided to them initially by autotrophs. Many organisms, ranging from humans to many prokaryotes, including the well-studied *Escherichia coli*, are heterotrophic.

Organisms can also be identified by the energy source they use. All energy is derived from the transfer of electrons, but the source of electrons differs between various types of organisms. The prefixes photo- ("light") and chemo- ("chemical") refer to the energy sources that various organisms use. Those that get their energy for electron transfer from light are **phototrophs**, whereas **chemotrophs** obtain energy for electron transfer by breaking chemical bonds. There are two types of chemotrophs: **organotrophs** and **lithotrophs**. Organotrophs, including humans, fungi, and many prokaryotes, are chemotrophs that obtain energy from organic compounds. Lithotrophs ("litho" means "rock") are chemotrophs that get energy from inorganic compounds, including hydrogen sulfide (H_2S) and reduced iron. Lithotrophy is unique to the microbial world.

The strategies used to obtain both carbon and energy can be combined for the classification of organisms according to nutritional type. Most organisms are chemoheterotrophs because they use organic molecules as both their electron and carbon sources. [\[link\]](#) summarizes this and the other classifications.

Classifications of Organisms by Energy and Carbon Source

Classifications	Energy Source	Carbon Source	Examples
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Classifications of Organisms by Energy and Carbon Source				
Classifications		Energy Source	Carbon Source	Examples
Chemotrophs	Chemoautotrophs	Chemical	Inorganic	Hydrogen-, sulfur-, iron-, nitrogen-, and carbon monoxide- oxidizing bacteria
	Chemoheterotrophs	Chemical	Organic compounds	All animals, most fungi, protozoa, and bacteria
Phototrophs	Photoautotrophs	Light	Inorganic	All plants, algae, cyanobacteria, and green and purple sulfur bacteria
	Photoheterotrophs	Light	Organic compounds	Green and purple nonsulfur bacteria, heliobacteria

Note:

- Explain the difference between catabolism and anabolism.
- Explain the difference between autotrophs and heterotrophs.

Oxidation and Reduction in Metabolism

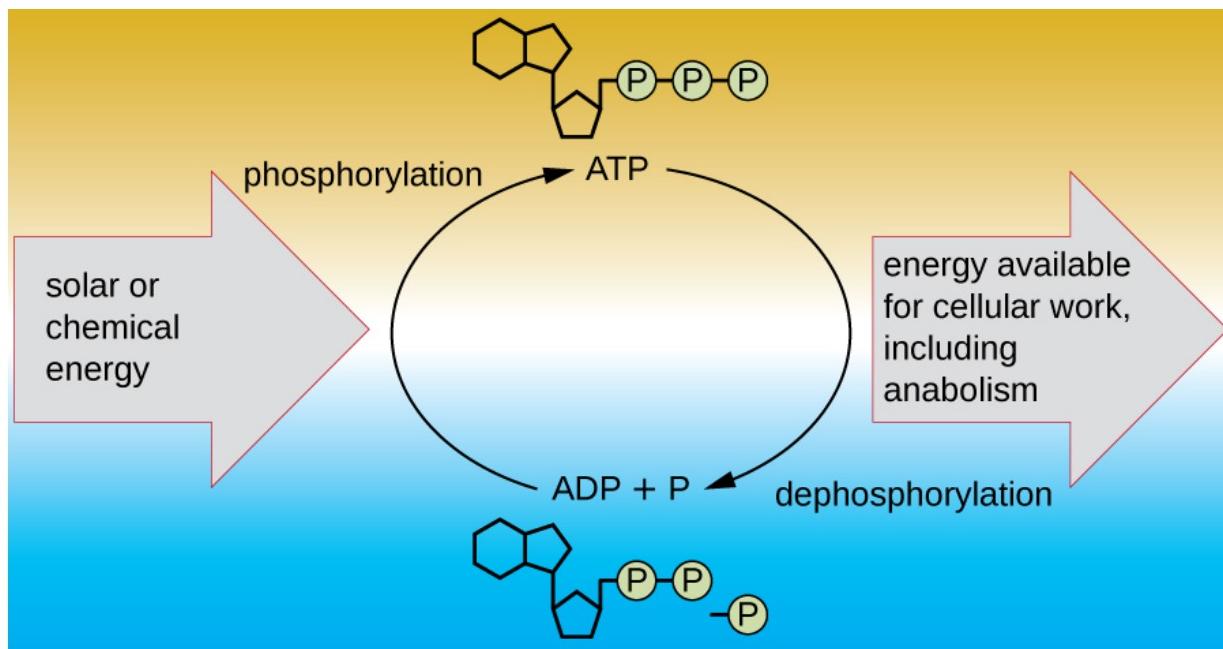
The transfer of electrons between molecules is important because most of the energy stored in atoms and used to fuel cell functions is in the form of high-energy electrons. The transfer of energy in the form of electrons allows the cell to transfer and use energy incrementally; that is,

in small packages rather than a single, destructive burst. Reactions that remove electrons from donor molecules, leaving them oxidized, are **oxidation reactions**; those that add electrons to acceptor molecules, leaving them reduced, are **reduction reactions**. Because electrons can move from one molecule to another, oxidation and reduction occur in tandem. These pairs of reactions are called oxidation-reduction reactions, or **redox reactions**.

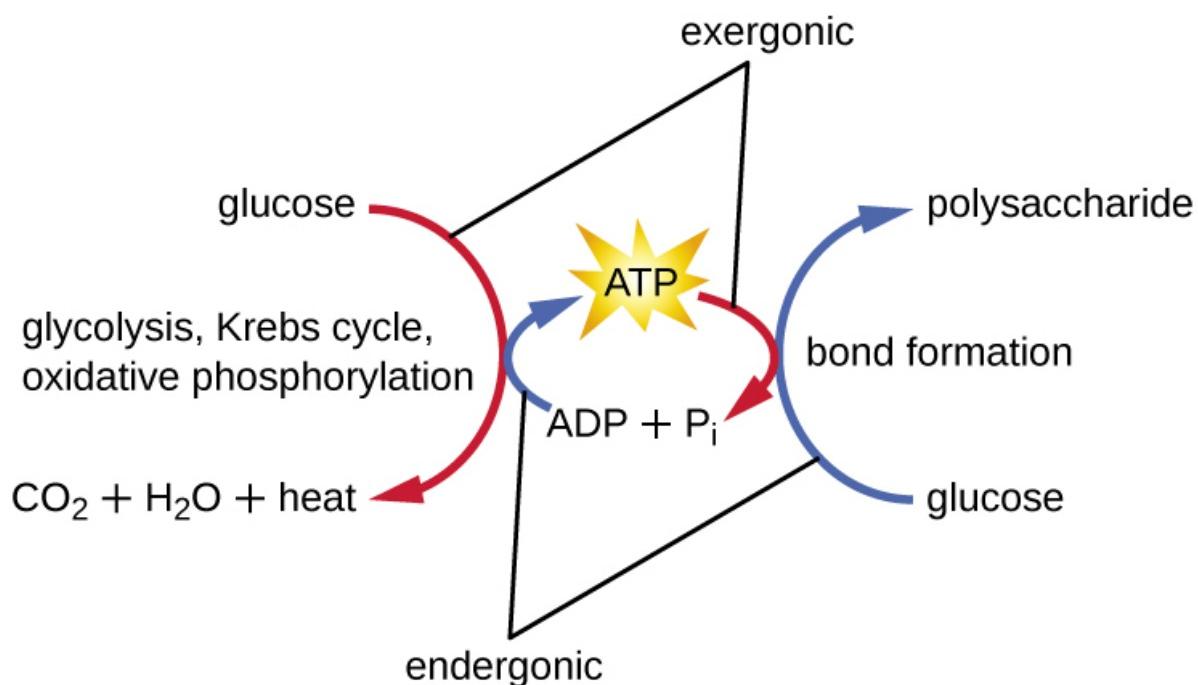
Energy Carriers: NAD⁺, NADP⁺, FAD, and ATP

The energy released from the breakdown of the chemical bonds within nutrients can be stored either through the reduction of electron carriers or in the bonds of adenosine triphosphate (ATP). In living systems, a small class of compounds functions as mobile **electron carriers**, molecules that bind to and shuttle high-energy electrons between compounds in pathways. The principal electron carriers we will consider originate from the B vitamin group and are derivatives of nucleotides; they are **nicotinamide adenine dinucleotide**, **nicotine adenine dinucleotide phosphate**, and **flavin adenine dinucleotide**. These compounds can be easily reduced or oxidized. Nicotinamide adenine dinucleotide (**NAD⁺/NADH**) is the most common mobile electron carrier used in catabolism. NAD⁺ is the oxidized form of the molecule; NADH is the reduced form of the molecule. Nicotine adenine dinucleotide phosphate (**NADP⁺**), the oxidized form of an NAD⁺ variant that contains an extra phosphate group, is another important electron carrier; it forms **NADPH** when reduced. The oxidized form of flavin adenine dinucleotide is **FAD**, and its reduced form is **FADH₂**. Both NAD⁺/NADH and FAD/FADH₂ are extensively used in energy extraction from sugars during catabolism in chemoheterotrophs, whereas NADP⁺/NADPH plays an important role in anabolic reactions and photosynthesis. Collectively, FADH₂, NADH, and NADPH are often referred to as having reducing power due to their ability to donate electrons to various chemical reactions.

A living cell must be able to handle the energy released during catabolism in a way that enables the cell to store energy safely and release it for use only as needed. Living cells accomplish this by using the compound **adenosine triphosphate (ATP)**. ATP is often called the “energy currency” of the cell, and, like currency, this versatile compound can be used to fill any energy need of the cell. At the heart of ATP is a molecule of **adenosine monophosphate (AMP)**, which is composed of an adenine molecule bonded to a ribose molecule and a single phosphate group. Ribose is a five-carbon sugar found in RNA, and AMP is one of the nucleotides in RNA. The addition of a second phosphate group to this core molecule results in the formation of **adenosine diphosphate (ADP)**; the addition of a third phosphate group forms ATP ([\[link\]](#)). Adding a phosphate group to a molecule, a process called phosphorylation, requires energy. Phosphate groups are negatively charged and thus repel one another when they are arranged in series, as they are in ADP and ATP. This repulsion makes the ADP and ATP molecules inherently unstable. Thus, the bonds between phosphate groups (one in ADP and two in ATP) are called **high-energy phosphate bonds**. When these high-energy bonds are broken to release one phosphate (called **inorganic phosphate [P_i]**) or two connected phosphate groups (called **pyrophosphate [PP_i]**) from ATP through a process called dephosphorylation, energy is released to drive endergonic reactions ([\[link\]](#)).



The energy released from dephosphorylation of ATP is used to drive cellular work, including anabolic pathways. ATP is regenerated through phosphorylation, harnessing the energy found in chemicals or from sunlight. (credit: modification of work by Robert Bear, David Rintoul)



Exergonic reactions are coupled to endergonic ones, making the combination favorable. Here, the endergonic reaction of ATP phosphorylation is coupled to the exergonic reactions of catabolism. Similarly, the exergonic reaction of ATP dephosphorylation is coupled to the endergonic reaction of polypeptide formation, an example of anabolism.

Note:

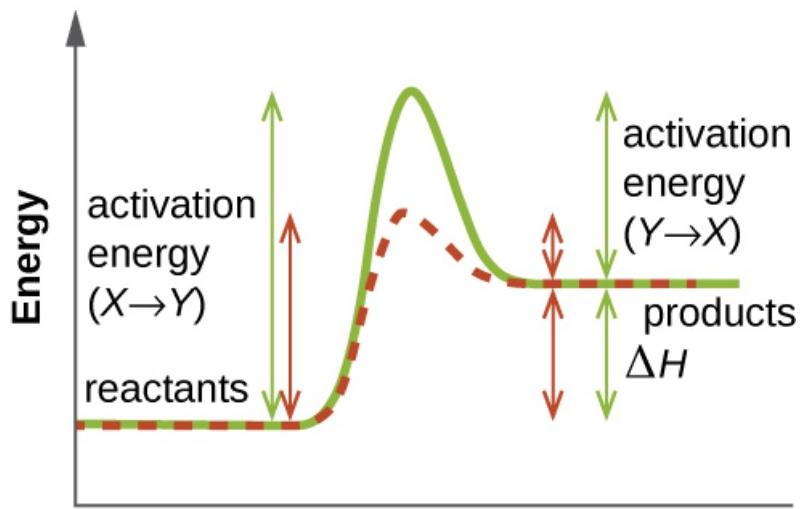
- What is the function of an electron carrier?

Enzyme Structure and Function

A substance that helps speed up a chemical reaction is a **catalyst**. Catalysts are not used or changed during chemical reactions and, therefore, are reusable. Whereas inorganic molecules may serve as catalysts for a wide range of chemical reactions, proteins called **enzymes** serve as catalysts for biochemical reactions inside cells. Enzymes thus play an important role in controlling cellular metabolism.

An enzyme functions by lowering the **activation energy** of a chemical reaction inside the cell. Activation energy is the energy needed to form or break chemical bonds and convert reactants to products ([\[link\]](#)). Enzymes lower the activation energy by binding to the reactant molecules and holding them in such a way as to speed up the reaction.

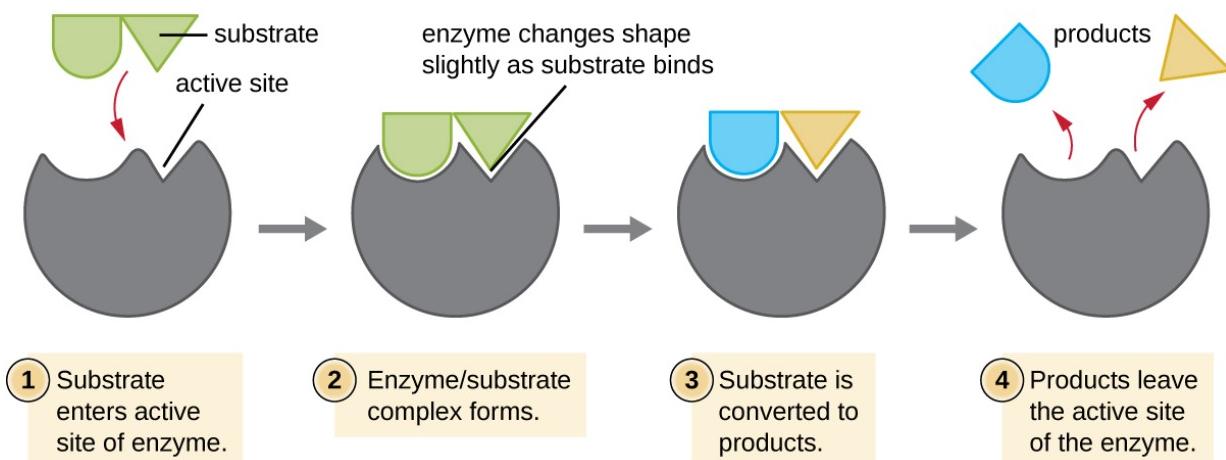
The chemical reactants to which an enzyme binds are called **substrates**, and the location within the enzyme where the substrate binds is called the enzyme's **active site**. The characteristics of the amino acids near the active site create a very specific chemical environment within the active site that induces suitability to binding, albeit briefly, to a specific substrate (or substrates). Due to this jigsaw puzzle-like match between an enzyme and its substrates, enzymes are known for their specificity. In fact, as an enzyme binds to its substrate(s), the enzyme structure changes slightly to find the best fit between the transition state (a structural intermediate between the substrate and product) and the active site, just as a rubber glove molds to a hand inserted into it. This active-site modification in the presence of substrate, along with the simultaneous formation of the transition state, is called induced fit ([\[link\]](#)). Overall, there is a specifically matched enzyme for each substrate and, thus, for each chemical reaction; however, there is some flexibility as well. Some enzymes have the ability to act on several different structurally related substrates.



Reaction path

- Reaction without catalyst
- - Reaction with catalyst

Enzymes lower the activation energy of a chemical reaction.



According to the induced-fit model, the active site of the enzyme undergoes conformational changes upon binding with the substrate.

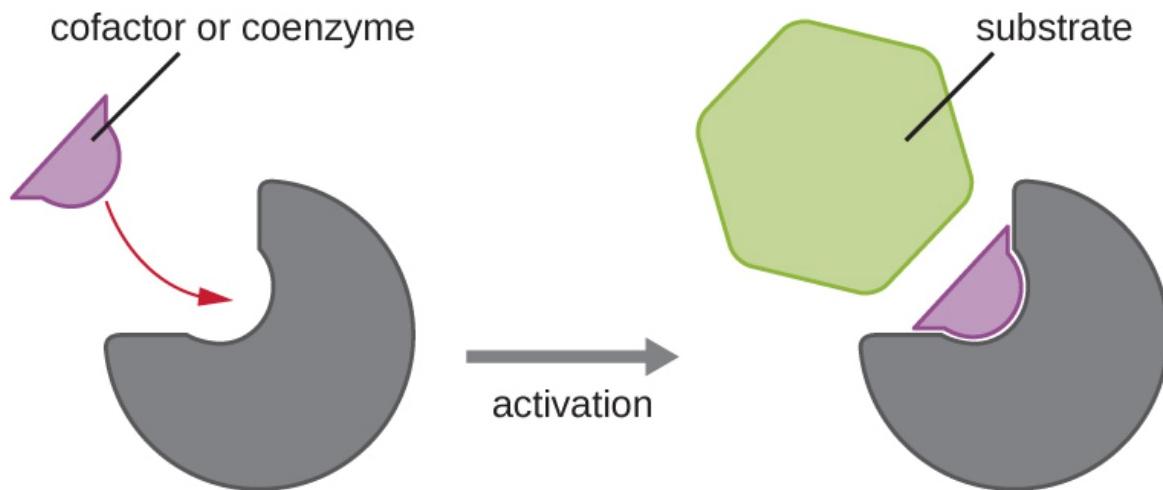
Enzymes are subject to influences by local environmental conditions such as pH, substrate concentration, and temperature. Although increasing the environmental temperature generally increases reaction rates, enzyme catalyzed or otherwise, increasing or decreasing the temperature outside of an optimal range can affect chemical bonds within the active site, making them less well suited to bind substrates. High temperatures will eventually cause enzymes, like other biological molecules, to denature, losing their three-dimensional structure and function. Enzymes are also suited to function best within a certain pH range, and, as with temperature, extreme environmental pH values (acidic or basic) can cause enzymes to denature. Active-site amino-acid side chains have their own acidic or basic properties that are optimal for catalysis and, therefore, are sensitive to changes in pH.

Another factor that influences enzyme activity is substrate concentration: Enzyme activity is increased at higher concentrations of substrate until it reaches a saturation point at which the enzyme can bind no additional substrate. Overall, enzymes are optimized to work best under the environmental conditions in which the organisms that produce them live. For example, while microbes that inhabit hot springs have enzymes that work best at high temperatures, human pathogens have enzymes that work best at 37°C. Similarly, while enzymes produced by most organisms work best at a neutral pH, microbes growing in acidic environments make enzymes optimized to low pH conditions, allowing for their growth at those conditions.

Many enzymes do not work optimally, or even at all, unless bound to other specific nonprotein helper molecules, either temporarily through ionic or hydrogen bonds or permanently through stronger covalent bonds. Binding to these molecules promotes optimal conformation and function for their respective enzymes. Two types of helper molecules are **cofactors** and **coenzymes**. Cofactors are inorganic ions such as iron (Fe^{2+}) and magnesium (Mg^{2+}) that help stabilize enzyme conformation and function. One example of an enzyme that requires a metal ion as a cofactor is the enzyme that builds DNA molecules, DNA polymerase, which requires a bound zinc ion (Zn^{2+}) to function.

Coenzymes are organic helper molecules that are required for enzyme action. Like enzymes, they are not consumed and, hence, are reusable. The most common sources of coenzymes are dietary vitamins. Some vitamins are precursors to coenzymes and others act directly as coenzymes.

Some cofactors and coenzymes, like coenzyme A (CoA), often bind to the enzyme's active site, aiding in the chemistry of the transition of a substrate to a product ([\[link\]](#)). In such cases, an enzyme lacking a necessary cofactor or coenzyme is called an **apoenzyme** and is inactive. Conversely, an enzyme with the necessary associated cofactor or coenzyme is called a **holoenzyme** and is active. NADH and ATP are also both examples of commonly used coenzymes that provide high-energy electrons or phosphate groups, respectively, which bind to enzymes, thereby activating them.



1 Apoenzyme becomes active by binding of coenzyme or cofactor to enzyme.

2 Holoenzyme is formed when associated cofactor or coenzyme binds to the enzyme's active site.

The binding of a coenzyme or cofactor to an apoenzyme is often required to form an active holoenzyme.

Note:

- What role do enzymes play in a chemical reaction?

Enzyme Inhibitors

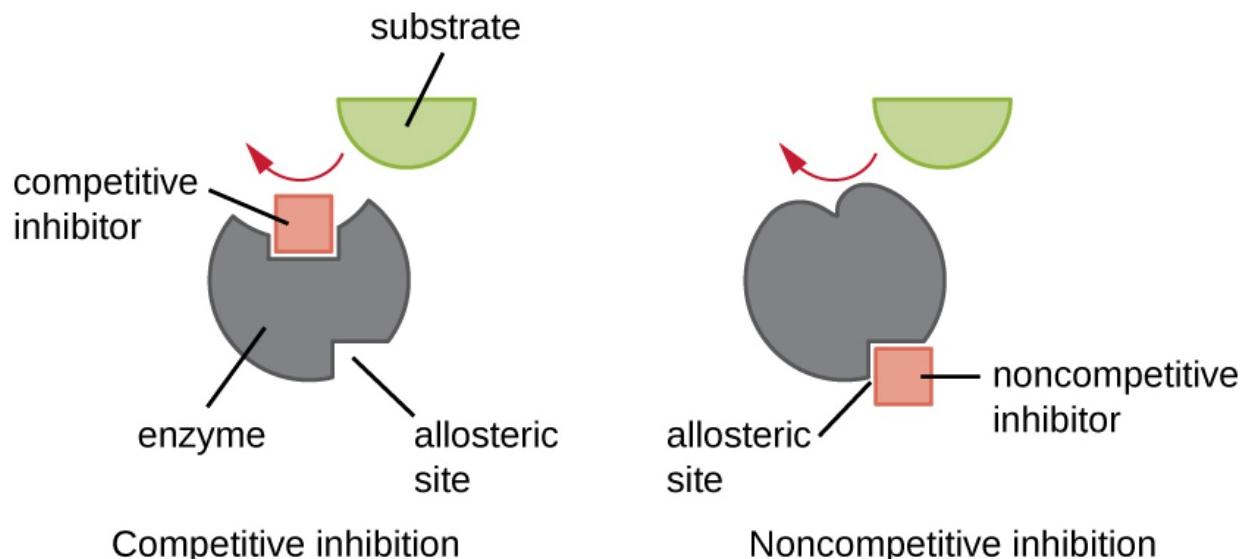
Enzymes can be regulated in ways that either promote or reduce their activity. There are many different kinds of molecules that inhibit or promote enzyme function, and various mechanisms exist for doing so ([\[link\]](#)). A **competitive inhibitor** is a molecule similar enough to a substrate that it can compete with the substrate for binding to the active site by simply blocking the substrate from binding. For a competitive inhibitor to be effective, the inhibitor concentration needs to be approximately equal to the substrate concentration. Sulfa drugs provide a good example of competitive competition. They are used to treat bacterial infections because they bind to the active site of an enzyme within the bacterial folic acid synthesis pathway. When present in a sufficient dose, a sulfa drug prevents folic acid synthesis, and bacteria are unable

to grow because they cannot synthesize DNA, RNA, and proteins. Humans are unaffected because we obtain folic acid from our diets.

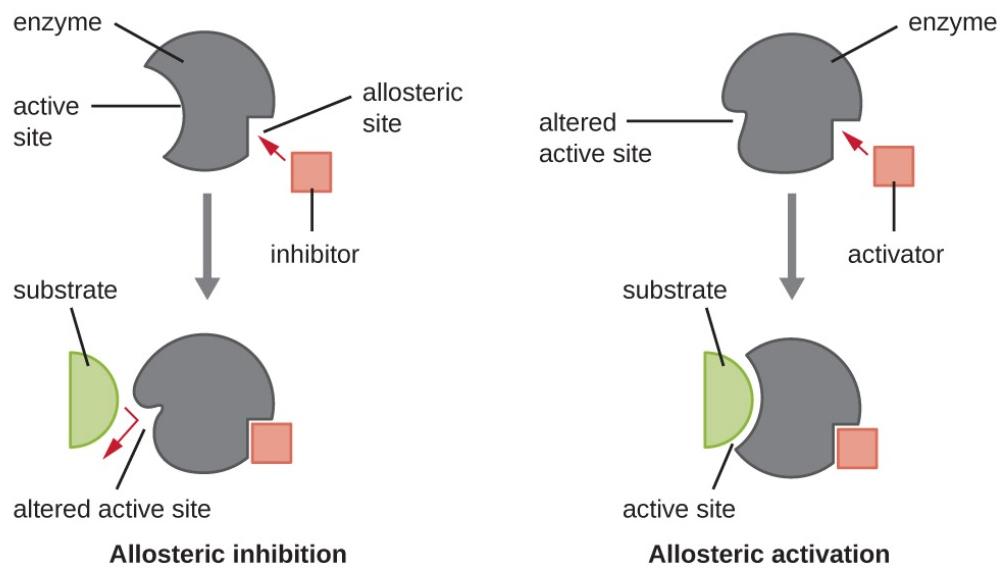
On the other hand, a **noncompetitive (allosteric) inhibitor** binds to the enzyme at an **allosteric site**, a location other than the active site, and still manages to block substrate binding to the active site by inducing a conformational change that reduces the affinity of the enzyme for its substrate ([\[link\]](#)). Because only one inhibitor molecule is needed per enzyme for effective inhibition, the concentration of inhibitors needed for noncompetitive inhibition is typically much lower than the substrate concentration.

In addition to allosteric inhibitors, there are **allosteric activators** that bind to locations on an enzyme away from the active site, inducing a conformational change that increases the affinity of the enzyme's active site(s) for its substrate(s).

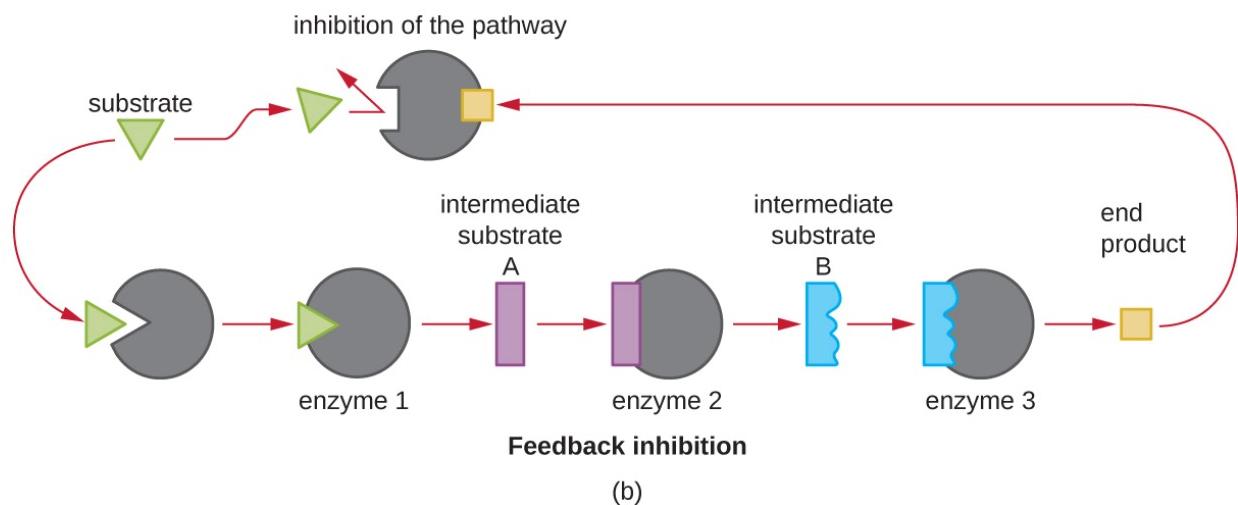
Allosteric control is an important mechanism of regulation of metabolic pathways involved in both catabolism and anabolism. In a most efficient and elegant way, cells have evolved also to use the products of their own metabolic reactions for **feedback inhibition** of enzyme activity. Feedback inhibition involves the use of a pathway product to regulate its own further production. The cell responds to the abundance of specific products by slowing production during anabolic or catabolic reactions ([\[link\]](#)).



Enzyme activity can be regulated by either competitive inhibitors, which bind to the active site, or noncompetitive inhibitors, which bind to an allosteric site.



(a)



Feedback inhibition

(b)

(a) Binding of an allosteric inhibitor reduces enzyme activity, but binding of an allosteric activator increases enzyme activity. (b) Feedback inhibition, where the end product of the pathway serves as a noncompetitive inhibitor to an enzyme early in the pathway, is an important mechanism of allosteric regulation in cells.

Note:

- Explain the difference between a competitive inhibitor and a noncompetitive inhibitor.

Key Concepts and Summary

- **Metabolism** includes chemical reactions that break down complex molecules (**catabolism**) and those that build complex molecules (**anabolism**).
- Organisms may be classified according to their source of carbon. **Autotrophs** convert inorganic carbon dioxide into organic carbon; **heterotrophs** use fixed organic carbon compounds.
- Organisms may also be classified according to their energy source. **Phototrophs** obtain their energy from light. **Chemotrophs** get their energy from chemical compounds. **Organotrophs** use organic molecules, and **lithotrophs** use inorganic chemicals.
- Cellular **electron carriers** accept high-energy electrons from foods and later serve as electron donors in subsequent **redox reactions**. **FAD/FADH₂, NAD⁺/NADH, and NADP⁺/NADPH** are important electron carriers.
- **Adenosine triphosphate (ATP)** serves as the energy currency of the cell, safely storing chemical energy in its two **high-energy phosphate bonds** for later use to drive processes requiring energy.
- **Enzymes** are biological **catalysts** that increase the rate of chemical reactions inside cells by lowering the activation energy required for the reaction to proceed.
- In nature, **exergonic reactions** do not require energy beyond activation energy to proceed, and they release energy. They may proceed without enzymes, but at a slow rate. Conversely, **endergonic reactions** require energy beyond activation energy to occur. In cells, endergonic reactions are coupled to exergonic reactions, making the combination energetically favorable.
- **Substrates** bind to the enzyme's **active site**. This process typically alters the structures of both the active site and the substrate, favoring transition-state formation; this is known as **induced fit**.
- **Cofactors** are inorganic ions that stabilize enzyme conformation and function. **Coenzymes** are organic molecules required for proper enzyme function and are often derived from vitamins. An enzyme lacking a cofactor or coenzyme is an **apoenzyme**; an enzyme with a bound cofactor or coenzyme is a **holoenzyme**.
- **Competitive inhibitors** regulate enzymes by binding to an enzyme's active site, preventing substrate binding. **Noncompetitive (allosteric) inhibitors** bind to **allosteric sites**, inducing a conformational change in the enzyme that prevents it from functioning. **Feedback inhibition** occurs when the product of a metabolic pathway noncompetitively binds to an enzyme early on in the pathway, ultimately preventing the synthesis of the product.

Short Answer

Exercise:

Problem:

In cells, can an oxidation reaction happen in the absence of a reduction reaction? Explain.

Exercise:

Problem:

What is the function of molecules like NAD⁺/NADH and FAD/FADH₂ in cells?

Catabolism of Carbohydrates

LEARNING OBJECTIVES

- Describe why glycolysis is not oxygen dependent
- Define and describe the net yield of three-carbon molecules, ATP, and NADH from glycolysis
- Explain how three-carbon pyruvate molecules are converted into two-carbon acetyl groups that can be funneled into the Krebs cycle.
- Define and describe the net yield of CO₂, GTP/ATP, FADH₂, and NADH from the Krebs cycle
- Explain how intermediate carbon molecules of the Krebs cycle can be used in a cell

Extensive enzyme pathways exist for breaking down carbohydrates to capture energy in ATP bonds. In addition, many catabolic pathways produce intermediate molecules that are also used as building blocks for anabolism. Understanding these processes is important for several reasons. First, because the main metabolic processes involved are common to a wide range of chemoheterotrophic organisms, we can learn a great deal about human metabolism by studying metabolism in more easily manipulated bacteria like *E. coli*. Second, because animal and human pathogens are also chemoheterotrophs, learning about the details of metabolism in these bacteria, including possible differences between bacterial and human pathways, is useful for the diagnosis of pathogens as well as for the discovery of antimicrobial therapies targeting specific pathogens. Last, learning specifically about the pathways involved in chemoheterotrophic metabolism also serves as a basis for comparing other more unusual

metabolic strategies used by microbes. Although the chemical source of electrons initiating electron transfer is different between chemoheterotrophs and chemoautotrophs, many similar processes are used in both types of organisms.

The typical example used to introduce concepts of metabolism to students is carbohydrate catabolism. For chemoheterotrophs, our examples of metabolism start with the catabolism of polysaccharides such as glycogen, starch, or cellulose. Enzymes such as amylase, which breaks down glycogen or starch, and cellulases, which break down cellulose, can cause the hydrolysis of glycosidic bonds between the glucose monomers in these polymers, releasing glucose for further catabolism.

Glycolysis

For bacteria, eukaryotes, and most archaea, **glycolysis** is the most common pathway for the catabolism of glucose; it produces energy, reduced electron carriers, and precursor molecules for cellular metabolism. Every living organism carries out some form of glycolysis, suggesting this mechanism is an ancient universal metabolic process. The process itself does not use oxygen; however, glycolysis can be coupled with additional metabolic processes that are either aerobic or anaerobic. Glycolysis takes place in the cytoplasm of prokaryotic and eukaryotic cells. It begins with a single six-carbon glucose molecule and ends with two molecules of a three-carbon sugar called pyruvate. Pyruvate may be broken down further after glycolysis to harness more energy through aerobic or anaerobic respiration, but many organisms, including many microbes, may be unable to respire; for these organisms, glycolysis may be their only source of generating ATP.

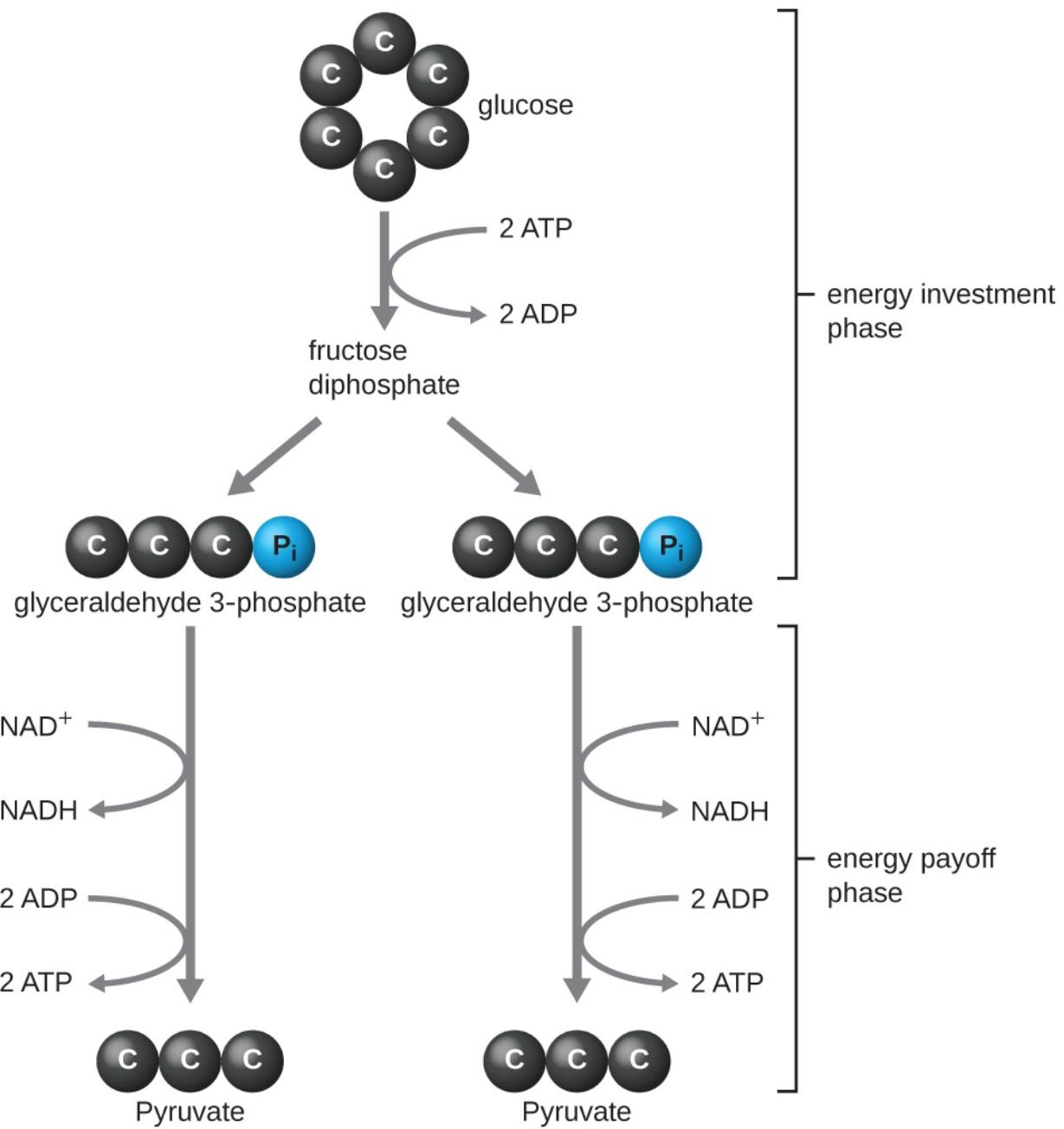
The type of glycolysis found in animals and that is most common in microbes is the **Embden-Meyerhof-Parnas (EMP) pathway**, named after Gustav Embden (1874–1933), Otto Meyerhof (1884–1951), and Jakub Parnas (1884–1949). Glycolysis using the EMP pathway consists of two distinct phases ([\[link\]](#)). The first part of the pathway, called the energy investment phase, uses energy from two ATP molecules to modify a glucose molecule so that the six-carbon sugar molecule can be split evenly into two phosphorylated three-carbon molecules called glyceraldehyde 3-phosphate

(G3P). The second part of the pathway, called the energy payoff phase, extracts energy by oxidizing G3P to pyruvate, producing four ATP molecules and reducing two molecules of NAD⁺ to two molecules of NADH, using electrons that originated from glucose. (A discussion and illustration of the full EMP pathway with chemical structures and enzyme names appear in [Appendix C](#).)

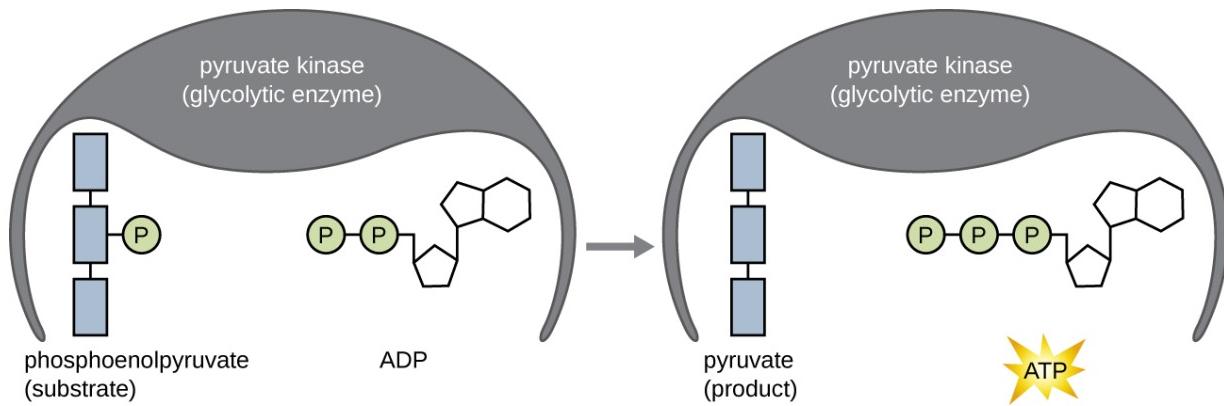
The ATP molecules produced during the energy payoff phase of glycolysis are formed by **substrate-level phosphorylation** ([\[link\]](#)), one of two mechanisms for producing ATP. In substrate-level phosphorylation, a phosphate group is removed from an organic molecule and is directly transferred to an available ADP molecule, producing ATP. During glycolysis, high-energy phosphate groups from the intermediate molecules are added to ADP to make ATP.

Overall, in this process of glycolysis, the net gain from the breakdown of a single glucose molecule is:

- two ATP molecules
- two NADH molecule, and
- two pyruvate molecules.



The energy investment phase of the Embden-Meyerhof-Parnas glycolysis pathway uses two ATP molecules to phosphorylate glucose, forming two glyceraldehyde 3-phosphate (G3P) molecules. The energy payoff phase harnesses the energy in the G3P molecules, producing four ATP molecules, two NADH molecules, and two pyruvates.



The ATP made during glycolysis is a result of substrate-level phosphorylation. One of the two enzymatic reactions in the energy payoff phase of Embden Meyerhof-Parnas glycolysis that produce ATP in this way is shown here.

Other Glycolytic Pathways

When we refer to glycolysis, unless otherwise indicated, we are referring to the EMP pathway used by animals and many bacteria. However, some prokaryotes use alternative glycolytic pathways. One important alternative is the **Entner-Doudoroff (ED) pathway**, named after its discoverers Nathan Entner and Michael Doudoroff (1911–1975). Although some bacteria, including the opportunistic gram-negative pathogen *Pseudomonas aeruginosa*, contain only the ED pathway for glycolysis, other bacteria, like *E. coli*, have the ability to use either the ED pathway or the EMP pathway.

A third type of glycolytic pathway that occurs in all cells, which is quite different from the previous two pathways, is the **pentose phosphate pathway (PPP)** also called the **phosphogluconate pathway** or the **hexose monophosphate shunt**. Evidence suggests that the PPP may be the most ancient universal glycolytic pathway. The intermediates from the PPP are used for the biosynthesis of nucleotides and amino acids. Therefore, this glycolytic pathway may be favored when the cell has need for nucleic acid and/or protein synthesis, respectively. A discussion and illustration of the

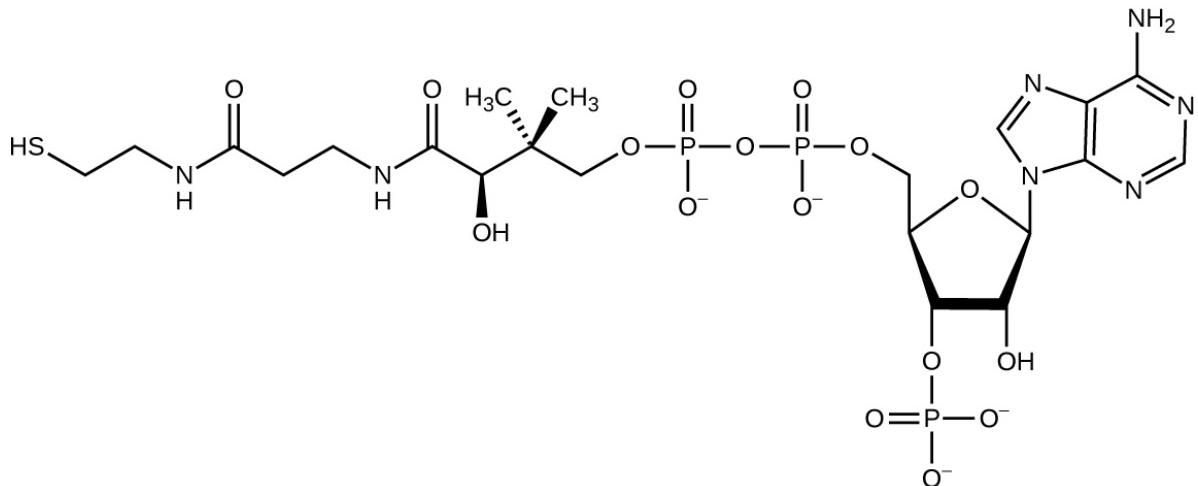
complete ED pathway and PPP with chemical structures and enzyme names appear in [Appendix C](#).

Note:

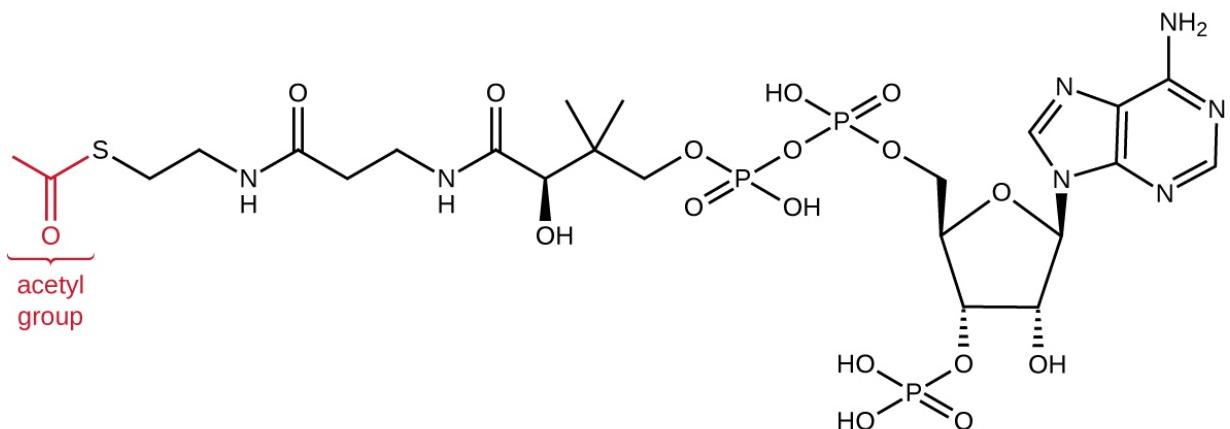
- When might an organism use the ED pathway or the PPP for glycolysis?

Transition Reaction, Coenzyme A, and the Krebs Cycle

Glycolysis produces pyruvate, which can be further oxidized to capture more energy. For pyruvate to enter the next oxidative pathway, it must first be decarboxylated by the enzyme complex pyruvate dehydrogenase to a two-carbon acetyl group in the **transition reaction**, also called the **bridge reaction** (see [Appendix C](#) and [\[link\]](#)). In the transition reaction, electrons are also transferred to NAD^+ to form NADH. To proceed to the next phase of this metabolic process, the comparatively tiny two-carbon acetyl must be attached to a very large carrier compound called coenzyme A (CoA). The transition reaction occurs in the mitochondrial matrix of eukaryotes; in prokaryotes, it occurs in the cytoplasm because prokaryotes lack membrane-enclosed organelles.



(a) coenzyme A without an attached acetyl group



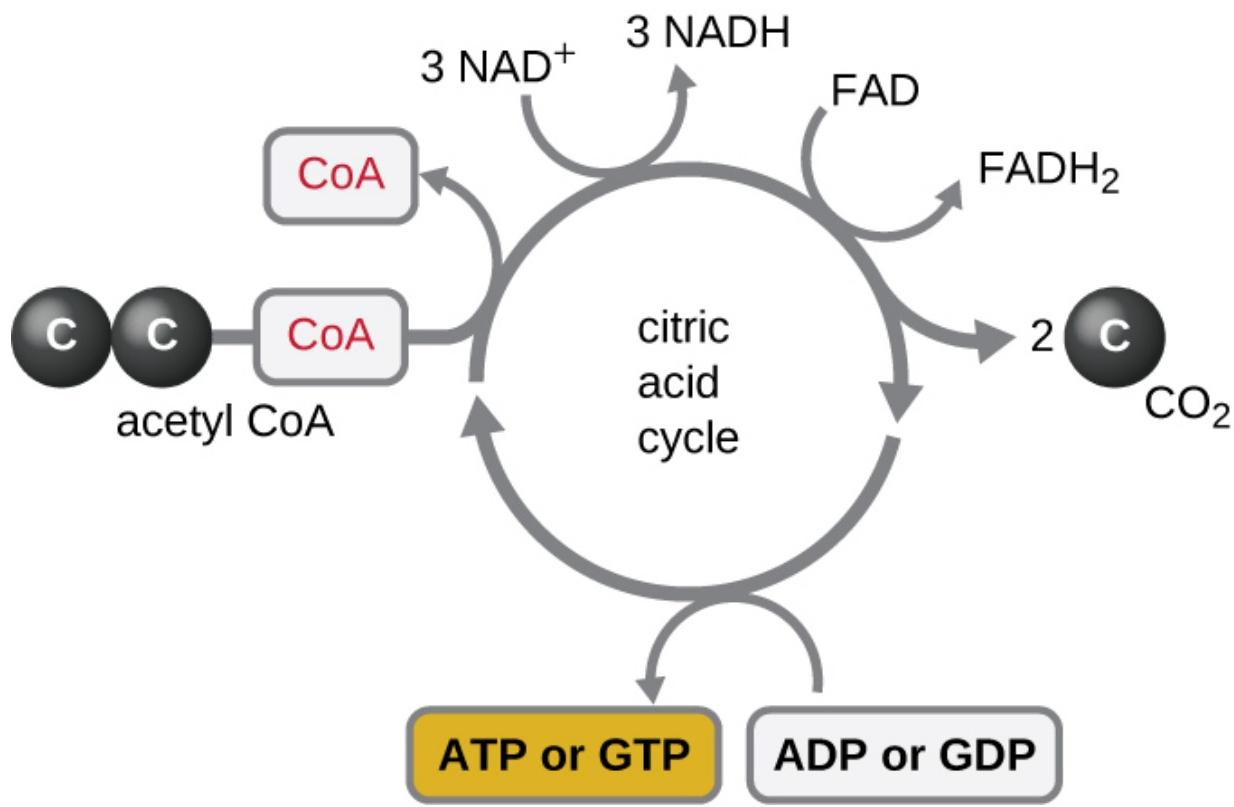
(b) coenzyme A with an attached acetyl group

- (a) Coenzyme A is shown here without an attached acetyl group. (b) Coenzyme A is shown here with an attached acetyl group.

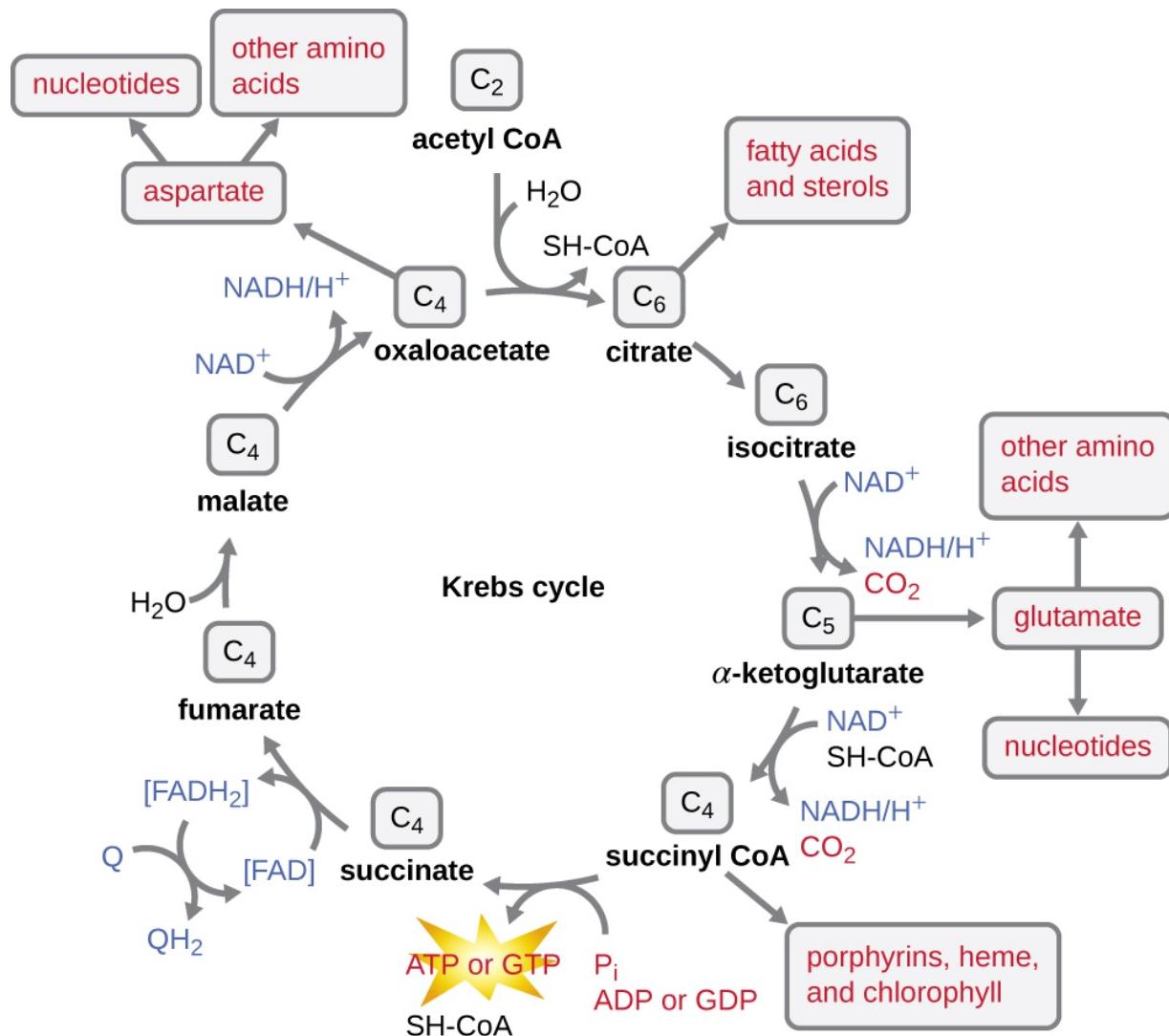
The **Krebs cycle** transfers remaining electrons from the acetyl group produced during the transition reaction to electron carrier molecules, thus reducing them. The Krebs cycle also occurs in the cytoplasm of prokaryotes along with glycolysis and the transition reaction, but it takes place in the mitochondrial matrix of eukaryotic cells where the transition reaction also occurs. The Krebs cycle is named after its discoverer, British scientist Hans Adolf Krebs (1900–1981) and is also called the **citric acid cycle**, or the **tricarboxylic acid cycle (TCA)** because citric acid has three carboxyl

groups in its structure. Unlike glycolysis, the Krebs cycle is a closed loop: The last part of the pathway regenerates the compound used in the first step ([\[link\]](#)). The eight steps of the cycle are a series of chemical reactions that capture the two-carbon acetyl group (the CoA carrier does not enter the Krebs cycle) from the transition reaction, which is added to a four-carbon intermediate in the Krebs cycle, producing the six-carbon intermediate citric acid (giving the alternate name for this cycle). As one turn of the cycle returns to the starting point of the four-carbon intermediate, the cycle produces two CO₂ molecules, one ATP molecule (or an equivalent, such as guanosine triphosphate [GTP]) produced by substrate-level phosphorylation, and three molecules of NADH and one of FADH₂. (A discussion and detailed illustration of the full Krebs cycle appear in [Appendix C.](#))

Although many organisms use the Krebs cycle as described as part of glucose metabolism, several of the intermediate compounds in the Krebs cycle can be used in synthesizing a wide variety of important cellular molecules, including amino acids, chlorophylls, fatty acids, and nucleotides; therefore, the cycle is both anabolic and catabolic ([\[link\]](#)).



The Krebs cycle, also known as the citric acid cycle, is summarized here. Note incoming two-carbon acetyl results in the main outputs per turn of two CO₂, three NADH, one FADH₂, and one ATP (or GTP) molecules made by substrate-level phosphorylation. Two turns of the Krebs cycle are required to process all of the carbon from one glucose molecule.



Many organisms use intermediates from the Krebs cycle, such as amino acids, fatty acids, and nucleotides, as building blocks for biosynthesis.

Key Concepts and Summary

- **Glycolysis** is the first step in the breakdown of glucose, resulting in the formation of ATP, which is produced by **substrate-level phosphorylation**; NADH; and two pyruvate molecules. Glycolysis does not use oxygen and is not oxygen dependent.

- After glycolysis, a three-carbon pyruvate is decarboxylated to form a two-carbon acetyl group, coupled with the formation of NADH. The acetyl group is attached to a large carrier compound called coenzyme A.
- After the transition step, coenzyme A transports the two-carbon acetyl to the **Krebs cycle**, where the two carbons enter the cycle. Per turn of the cycle, one acetyl group derived from glycolysis is further oxidized, producing three NADH molecules, one FADH₂, and one ATP by **substrate-level phosphorylation**, and releasing two CO₂ molecules.
- The Krebs cycle may be used for other purposes. Many of the intermediates are used to synthesize important cellular molecules, including amino acids, chlorophylls, fatty acids, and nucleotides.

Short Answer

Exercise:

Problem:

What is substrate-level phosphorylation? When does it occur during the breakdown of glucose to CO₂?

Exercise:

Problem:

Why is the Krebs cycle important in both catabolism and anabolism?

Critical Thinking

Exercise:

Problem:

What would be the consequences to a cell of having a mutation that knocks out coenzyme A synthesis?

Cellular Respiration

LEARNING OBJECTIVES

- Compare and contrast the electron transport system location and function in a prokaryotic cell and a eukaryotic cell
- Compare and contrast the differences between substrate-level and oxidative phosphorylation
- Explain the relationship between chemiosmosis and proton motive force
- Describe the function and location of ATP synthase in a prokaryotic versus eukaryotic cell
- Compare and contrast aerobic and anaerobic respiration

We have just discussed two pathways in glucose catabolism—glycolysis and the Krebs cycle—that generate ATP by substrate-level phosphorylation. Most ATP, however, is generated during a separate process called **oxidative phosphorylation**, which occurs during cellular respiration. Cellular respiration begins when electrons are transferred from NADH and FADH₂—made in glycolysis, the transition reaction, and the Krebs cycle—through a series of chemical reactions to a final inorganic electron acceptor (either oxygen in aerobic respiration or non-oxygen inorganic molecules in anaerobic respiration). These electron transfers take place on the inner part of the cell membrane of prokaryotic cells or in specialized protein complexes in the inner membrane of the mitochondria of eukaryotic cells. The energy of the electrons is harvested to generate an electrochemical gradient across the membrane, which is used to make ATP by oxidative phosphorylation.

Electron Transport System

The **electron transport system (ETS)** is the last component involved in the process of cellular respiration; it comprises a series of membrane-associated protein complexes and associated mobile accessory electron carriers ([\[link\]](#)). Electron transport is a series of chemical reactions that resembles a bucket brigade in that electrons from NADH and FADH₂ are passed rapidly from one ETS electron carrier to the next. These carriers can pass electrons along in the ETS because of their **redox potential**. For a protein or chemical to accept electrons, it must have a more positive redox potential than the electron donor. Therefore, electrons move from electron carriers with more negative redox potential to those with more positive redox potential. The four major classes of electron carriers involved in both eukaryotic and prokaryotic electron transport systems are the cytochromes, flavoproteins, iron-sulfur proteins, and the quinones.

In **aerobic respiration**, the final electron acceptor (i.e., the one having the most positive redox potential) at the end of the ETS is an oxygen molecule (O₂) that becomes reduced to water (H₂O) by the final ETS carrier. This electron carrier, **cytochrome oxidase**, differs between bacterial types and can be used to differentiate closely related bacteria for diagnoses. For example, the gram-negative opportunist *Pseudomonas aeruginosa* and the gram-negative cholera-causing *Vibrio cholerae* use cytochrome c oxidase, which can be detected by the oxidase test, whereas other gram-negative Enterobacteriaceae, like *E. coli*, are negative for this test because they produce different cytochrome oxidase types.

There are many circumstances under which aerobic respiration is not possible, including any one or more of the following:

- The cell lacks genes encoding an appropriate cytochrome oxidase for transferring electrons to oxygen at the end of the electron transport system.
- The cell lacks genes encoding enzymes to minimize the severely damaging effects of dangerous oxygen radicals produced during aerobic respiration, such as hydrogen peroxide (H₂O₂) or superoxide (O₂⁻).

- The cell lacks a sufficient amount of oxygen to carry out aerobic respiration.

One possible alternative to aerobic respiration is **anaerobic respiration**, using an inorganic molecule other than oxygen as a final electron acceptor. There are many types of anaerobic respiration found in bacteria and archaea. Denitrifiers are important soil bacteria that use nitrate (NO_3^-) and nitrite (NO_2^-) as final electron acceptors, producing nitrogen gas (N_2). Many aerobically respiring bacteria, including *E. coli*, switch to using nitrate as a final electron acceptor and producing nitrite when oxygen levels have been depleted.

Microbes using anaerobic respiration commonly have an intact Krebs cycle, so these organisms can access the energy of the NADH and FADH_2 molecules formed. However, anaerobic respirers use altered ETS carriers encoded by their genomes, including distinct complexes for electron transfer to their final electron acceptors. Smaller electrochemical gradients are generated from these electron transfer systems, so less ATP is formed through anaerobic respiration.

Note:

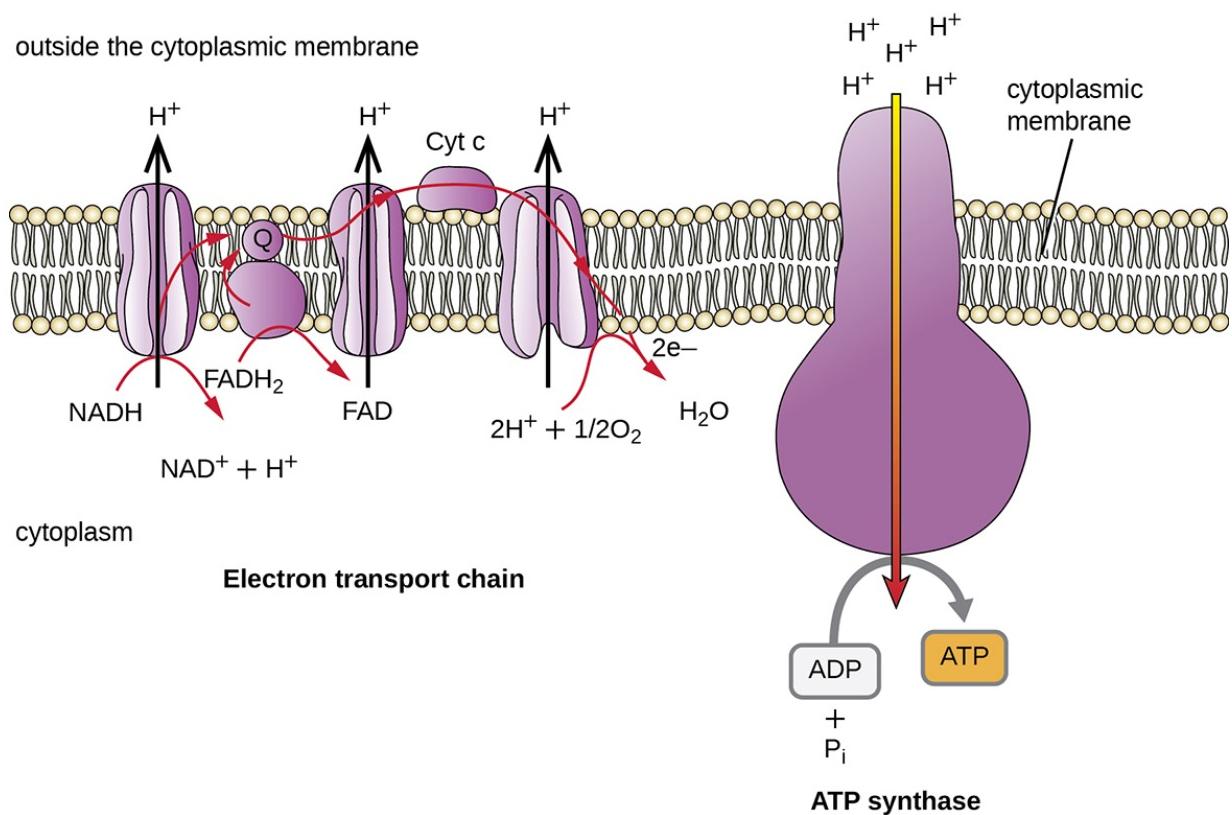
- Do both aerobic respiration and anaerobic respiration use an electron transport chain?

Chemiosmosis, Proton Motive Force, and Oxidative Phosphorylation

In each transfer of an electron through the ETS, the electron loses energy, but with some transfers, the energy is stored as potential energy by using it to pump hydrogen ions (H^+) across a membrane. In prokaryotic cells, H^+ is pumped to the outside of the cytoplasmic membrane (called the periplasmic space in gram-negative and gram-positive bacteria), and in eukaryotic cells,

they are pumped from the mitochondrial matrix across the inner mitochondrial membrane into the intermembrane space. There is an uneven distribution of H⁺ across the membrane that establishes an electrochemical gradient because H⁺ ions are positively charged (electrical) and there is a higher concentration (chemical) on one side of the membrane. This electrochemical gradient formed by the accumulation of H⁺ (also known as a proton) on one side of the membrane compared with the other is referred to as the **proton motive force** (PMF). Because the ions involved are H⁺, a pH gradient is also established, with the side of the membrane having the higher concentration of H⁺ being more acidic. Beyond the use of the PMF to make ATP, as discussed in this chapter, the PMF can also be used to drive other energetically unfavorable processes, including nutrient transport and flagella rotation for motility.

The potential energy of this electrochemical gradient generated by the ETS causes the H⁺ to diffuse across a membrane (the plasma membrane in prokaryotic cells and the inner membrane in mitochondria in eukaryotic cells). This flow of hydrogen ions across the membrane, called **chemiosmosis**, must occur through a channel in the membrane via a membrane-bound enzyme complex called **ATP synthase** ([\[link\]](#)). The tendency for movement in this way is much like water accumulated on one side of a dam, moving through the dam when opened. ATP synthase (like a combination of the intake and generator of a hydroelectric dam) is a complex protein that acts as a tiny generator, turning by the force of the H⁺ diffusing through the enzyme, down their electrochemical gradient from where there are many mutually repelling H⁺ to where there are fewer H⁺. In prokaryotic cells, H⁺ flows from the outside of the cytoplasmic membrane into the cytoplasm, whereas in eukaryotic mitochondria, H⁺ flows from the intermembrane space to the mitochondrial matrix. The turning of the parts of this molecular machine regenerates ATP from ADP and inorganic phosphate (P_i) by oxidative phosphorylation, a second mechanism for making ATP that harvests the potential energy stored within an electrochemical gradient.



The bacterial electron transport chain is a series of protein complexes, electron carriers, and ion pumps that is used to pump H⁺ out of the bacterial cytoplasm into the extracellular space. H⁺ flows back down the electrochemical gradient into the bacterial cytoplasm through ATP synthase, providing the energy for ATP production by oxidative phosphorylation.(credit: modification of work by Klaus Hoffmeier)

The number of ATP molecules generated from the catabolism of glucose varies. For example, the number of hydrogen ions that the electron transport system complexes can pump through the membrane varies between different species of organisms. In aerobic respiration in mitochondria, the passage of electrons from one molecule of NADH generates enough proton motive force to make three ATP molecules by oxidative phosphorylation, whereas the passage of electrons from one molecule of FADH₂ generates enough proton motive force to make only two ATP molecules. Thus, the 10 NADH molecules made per glucose during glycolysis, the transition

reaction, and the Krebs cycle carry enough energy to make 30 ATP molecules, whereas the two FADH₂ molecules made per glucose during these processes provide enough energy to make four ATP molecules. Overall, the theoretical maximum yield of ATP made during the complete aerobic respiration of glucose is 38 molecules, with four being made by substrate-level phosphorylation and 34 being made by oxidative phosphorylation ([\[link\]](#)). In reality, the total ATP yield is usually less, ranging from one to 34 ATP molecules, depending on whether the cell is using aerobic respiration or anaerobic respiration; in eukaryotic cells, some energy is expended to transport intermediates from the cytoplasm into the mitochondria, affecting ATP yield.

[\[link\]](#) summarizes the theoretical maximum yields of ATP from various processes during the complete aerobic respiration of one glucose molecule.

Source	Carbon Flow	Molecules of Reduced Coenzymes Produced	Net ATP Molecules Made by Substrate-Level Phosphorylation	Net ATP Molecules Made by Oxidative Phosphorylation	Theoretical Maximum Yield of ATP Molecules
Glycolysis (EMP)	Glucose (6C) → 2 pyruvates (3C)	2 NADH	2 ATP	6 ATP from 2 NADH	8
Transition reaction	2 pyruvates (3C) → 2 acetyl (2C) + 2 CO ₂	2 NADH		6 ATP from 2 NADH	6
Krebs cycle	2 acetyl (2C) → 4 CO ₂	6 NADH 2 FADH ₂	2 ATP	18 ATP from 6 NADH 4 ATP from 2 FADH ₂	24
Total:	glucose (6C) → 6 CO ₂	10 NADH 2 FADH ₂	4 ATP	34 ATP	38 ATP

Note:

- What are the functions of the proton motive force?

Key Concepts and Summary

- Most ATP generated during the cellular respiration of glucose is made by **oxidative phosphorylation**.
- An **electron transport system (ETS)** is composed of a series of membrane-associated protein complexes and associated mobile accessory electron carriers. The ETS is embedded in the cytoplasmic membrane of prokaryotes and the inner mitochondrial membrane of eukaryotes.
- Each ETS complex has a different redox potential, and electrons move from electron carriers with more negative redox potential to those with more positive redox potential.
- To carry out **aerobic respiration**, a cell requires oxygen as the final electron acceptor. A cell also needs a complete Krebs cycle, an appropriate cytochrome oxidase, and oxygen detoxification enzymes to prevent the harmful effects of oxygen radicals produced during aerobic respiration.
- Organisms performing **anaerobic respiration** use alternative electron transport system carriers for the ultimate transfer of electrons to the final non-oxygen electron acceptors.
- Microbes show great variation in the composition of their electron transport systems, which can be used for diagnostic purposes to help identify certain pathogens.
- As electrons are passed from NADH and FADH₂ through an ETS, the electron loses energy. This energy is stored through the pumping of H⁺ across the membrane, generating a **proton motive force**.
- The energy of this proton motive force can be harnessed by allowing hydrogen ions to diffuse back through the membrane by **chemiosmosis** using **ATP synthase**. As hydrogen ions diffuse through down their electrochemical gradient, components of ATP synthase spin, making ATP from ADP and P_i by oxidative phosphorylation.

- Aerobic respiration forms more ATP (a maximum of 34 ATP molecules) during oxidative phosphorylation than does anaerobic respiration (between one and 32 ATP molecules).

Multiple Choice

Exercise:

Problem:

Which is the location of electron transports systems in prokaryotes?

- A. the outer mitochondrial membrane
- B. the cytoplasm
- C. the inner mitochondrial membrane
- D. the cytoplasmic membrane

Solution:

D

Exercise:

Problem:

Which is the source of the energy used to make ATP by oxidative phosphorylation?

- A. oxygen
- B. high-energy phosphate bonds
- C. the proton motive force
- D. P_i

Solution:

C

Exercise:

Problem:

A cell might perform anaerobic respiration for which of the following reasons?

- A. It lacks glucose for degradation.
 - B. It lacks the transition reaction to convert pyruvate to acetyl-CoA.
 - C. It lacks Krebs cycle enzymes for processing acetyl-CoA to CO₂.
 - D. It lacks a cytochrome oxidase for passing electrons to oxygen.
-

Solution:

D

Exercise:

Problem: In prokaryotes, which of the following is true?

- A. As electrons are transferred through an ETS, H⁺ is pumped out of the cell.
 - B. As electrons are transferred through an ETS, H⁺ is pumped into the cell.
 - C. As protons are transferred through an ETS, electrons are pumped out of the cell.
 - D. As protons are transferred through an ETS, electrons are pumped into the cell.
-

Solution:

A

Exercise:

Problem:

Which of the following is not an electron carrier within an electron transport system?

- A. flavoprotein
 - B. ATP synthase
 - C. ubiquinone
 - D. cytochrome oxidase
-

Solution:

B

Fill in the Blank

Exercise:

Problem:

The final ETS complex used in aerobic respiration that transfers energy-depleted electrons to oxygen to form H₂O is called _____.

Solution:

cytochrome oxidase

Exercise:

Problem:

The passage of hydrogen ions through _____ down their electrochemical gradient harnesses the energy needed for ATP synthesis by oxidative phosphorylation.

Solution:

ATP synthase

True/False

Exercise:

Problem:

All organisms that use aerobic cellular respiration have cytochrome oxidase.

Solution:

True

Short Answer**Exercise:****Problem:**

What is the relationship between chemiosmosis and the proton motive force?

Exercise:**Problem:**

How does oxidative phosphorylation differ from substrate-level phosphorylation?

Exercise:**Problem:**

How does the location of ATP synthase differ between prokaryotes and eukaryotes? Where do protons accumulate as a result of the ETS in each cell type?

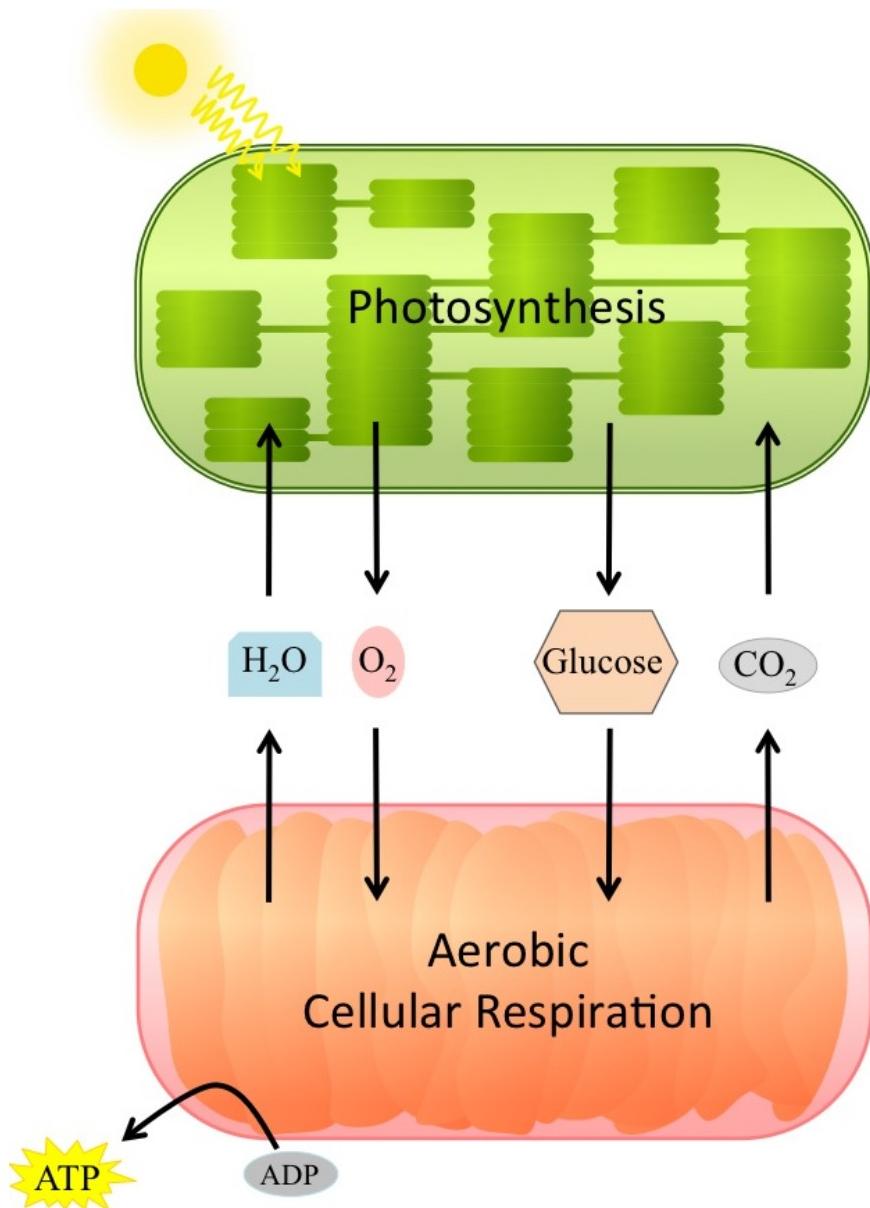
Overview of Cellular Respiration

This is a general overview of cellular respiration.

Introduction

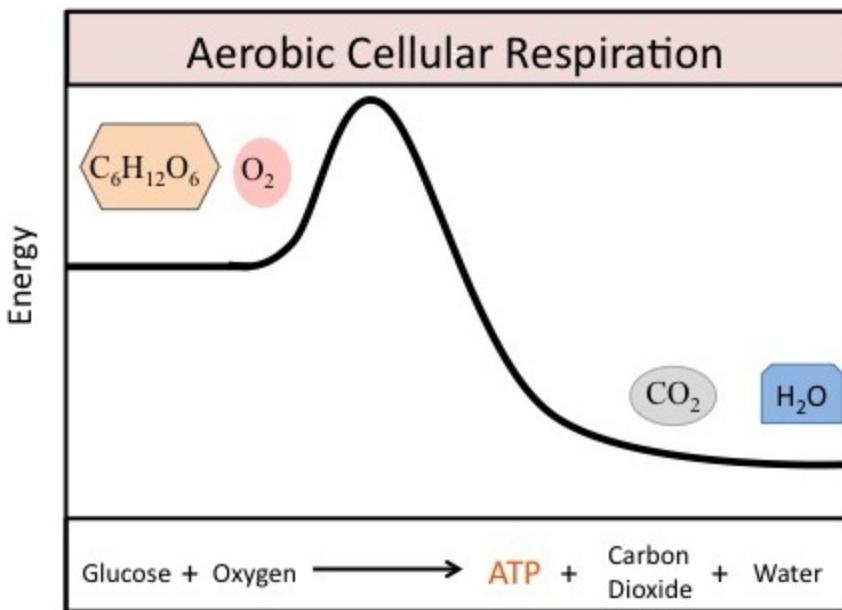
"Surely the mitochondrion that first entered another cell was not thinking about the future benefits of cooperation and integration; it was merely trying to make its own living in a tough Darwinian world." Stephen Jay Gould, in *Wonderful Life: the Burgess Shale and the Nature of History*, (1990)

All living organisms require energy, and for all organisms this energy comes from the chemical energy found in compounds that they acquire from their environment. The mitochondrion, a descendent of an aerobically-respiring bacteria, is the site of energy generation in eukaryotes. As we learned previously, the process of photosynthesis uses solar energy (sunlight) and converts this energy into chemical energy in the form of carbohydrates. In order for the chemical energy in the carbohydrates to be made available to do cellular work, the energy must be converted into a useable form known as ATP. Adenosine Triphosphate is the energy currency of the cell, and everything you do from walking down the street to reading this book requires energy in the form of ATP. Organisms need a constant supply of ATP, and the potential energy stored in food is the source of energy to meet this need. By connecting all this together, you should realize that your daily activities are fueled by the energy from the sun and that even on the cellular level nutrients cycle and energy flows ([\[link\]](#)).



This image illustrates the relationship between photosynthesis and cellular respiration. (Image by Eva Horne and Robert Bear)

All organisms need ATP, but not all organisms use the same pathways to generate ATP from the food that is consumed. **Aerobic cellular respiration**, the main subject of this chapter, uses oxygen (O_2) and glucose to generate ATP. Organisms (plants, animals, fungi and microbes) that live in an oxygen (O_2) rich environment use this process to generate ATP. The overall equation for aerobic cellular respiration is the reverse of photosynthesis, is an exergonic reaction, and supplies the ATP for cellular functions ([\[link\]](#)).



This image illustrates the overall equation for aerobic cellular respiration and how the amounts of free energy differs between the reactants and the products. (Image by Robert Bear)

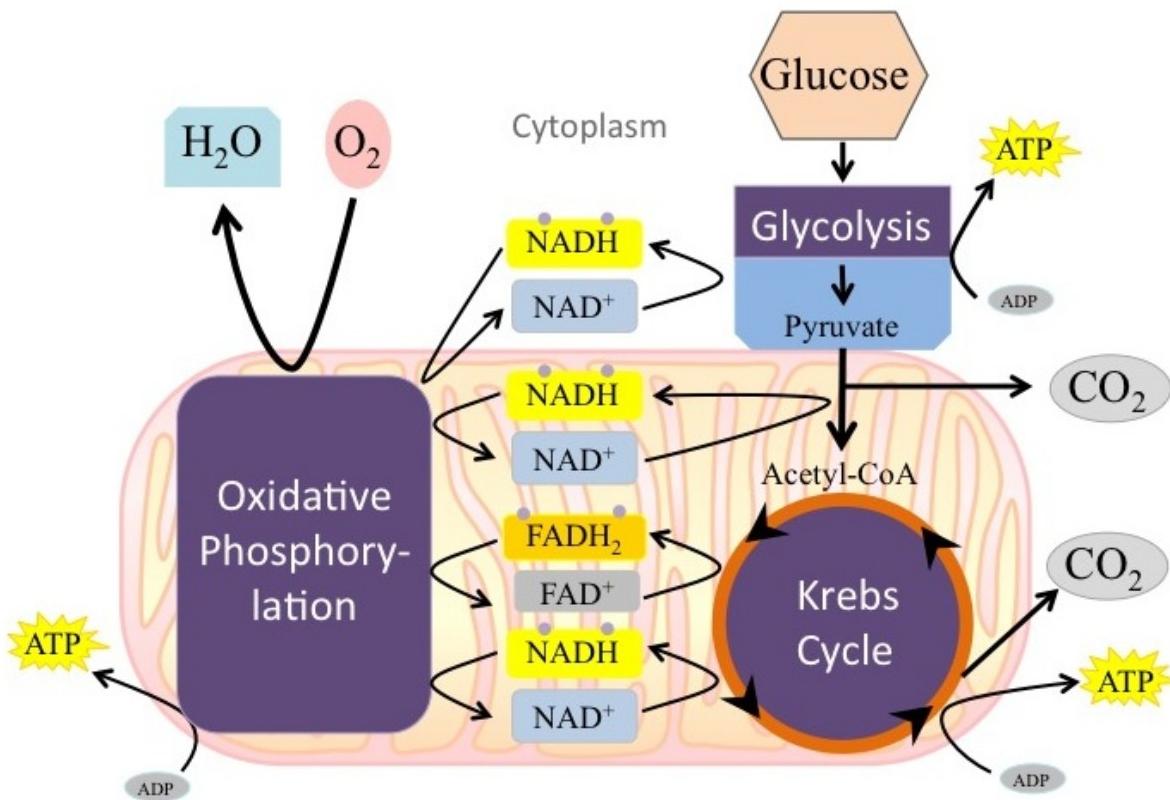
As the aerobic cellular respiration equation shows ([\[link\]](#)), an organism needs to acquire the O_2 from its surroundings and to get rid of the CO_2 that is produced. The acquisition of O_2 and the release of CO_2 is accomplished in a variety of ways. In single celled organisms, the movement of O_2 and CO_2 (gas exchange) is done by simple diffusion. However, in complex organisms there are specialized organs that allow for gas exchange; for example, gills in aquatic organisms and lungs in terrestrial animals.

A common misconception is that plants do not undergo cellular respiration because they make their own energy by photosynthesis. Plants do perform cellular respiration using the carbohydrates produced via photosynthesis; this occurs in tissues that are not photosynthetically active (e.g., roots), as well as in leaves and stems. Approximately half of the glucose produced by photosynthesis is consumed by the plant, mostly to generate ATP during aerobic cellular respiration. Other uses of glucose in the plant include synthesis of cell walls, starch, and other plant carbohydrates. So, plants harvest light energy via photosynthesis, making carbohydrates, and then they use the energy stored in those carbohydrates to perform various cellular functions. This is the reason why they are called **autotrophs**, or self feeders.

Some single-celled organisms use **anaerobic metabolism** to extract energy from biological molecules; this process occurs in the absence of oxygen. In this chapter, we will explore one type of anaerobic metabolism called fermentation. You may already be familiar with a one type of fermentation, lactic acid fermentation, especially if you have recently over-exerted your muscles. Anaerobic metabolism is used by many organisms to produce ATP when oxygen is not available and thus pathways which require oxygen cannot be used. The amount of ATP produced by fermentation is much less than that produced by aerobic cellular respiration, so there is a cost and benefit associated with organisms utilizing fermentation.

Summary of Aerobic Cellular Respiration

Aerobic cellular respiration ([\[link\]](#)) is series of linked chemical reactions that can be best understood if it is separated into four stages. These are **glycolysis**, pyruvate oxidation, the **Krebs Cycle**, and **oxidative phosphorylation**. Similar to photosynthesis, cellular respiration uses a series of oxidation-reduction reactions. During these reactions, electrons are stripped from the chemical bonds of the original glucose molecule and eventually added to oxygen, via a series of intermediate steps. This series of reactions releases small amounts of energy at each step; this energy is used to drive the formation of ATP. This section is a brief introduction to the stages of aerobic respiration with more detail to follow in the chapter.



Aerobic Cellular Respiration

This image illustrates aerobic cellular respiration. (Image by Eva Horne and Robert Bear)

The first stage of cellular respiration is called **Glycolysis** and occurs in the cytoplasm of the cell. During glycolysis, 1 glucose molecule (with 6 carbon atoms) is broken down into 2 pyruvate molecules (with three carbon atoms each). This is accompanied by the production of a few ATP molecules and the storage of some high-energy electrons on the electron carrier NADH. Note that no O₂ is needed for this set of reactions, which means that glycolysis can proceed in the absence of oxygen.

The second stage is a short series of reactions called the **oxidation of pyruvate** during which pyruvate (3 carbon atoms) is converted to acetyl-CoA (two carbon atoms), accompanied by the production of CO₂ (one

carbon atom). This process occurs on the mitochondrial inner membrane, and as a result the acetyl-CoA is formed inside the mitochondria. Pyruvate is made in the cytoplasm, and this step moves the next compound in the pathway into the mitochondria. This is critical, since all subsequent steps in the pathway occur within the mitochondria. The other important event of this stage is the addition of high-energy electrons to NAD^+ , generating another molecule of the electron carrier NADH.

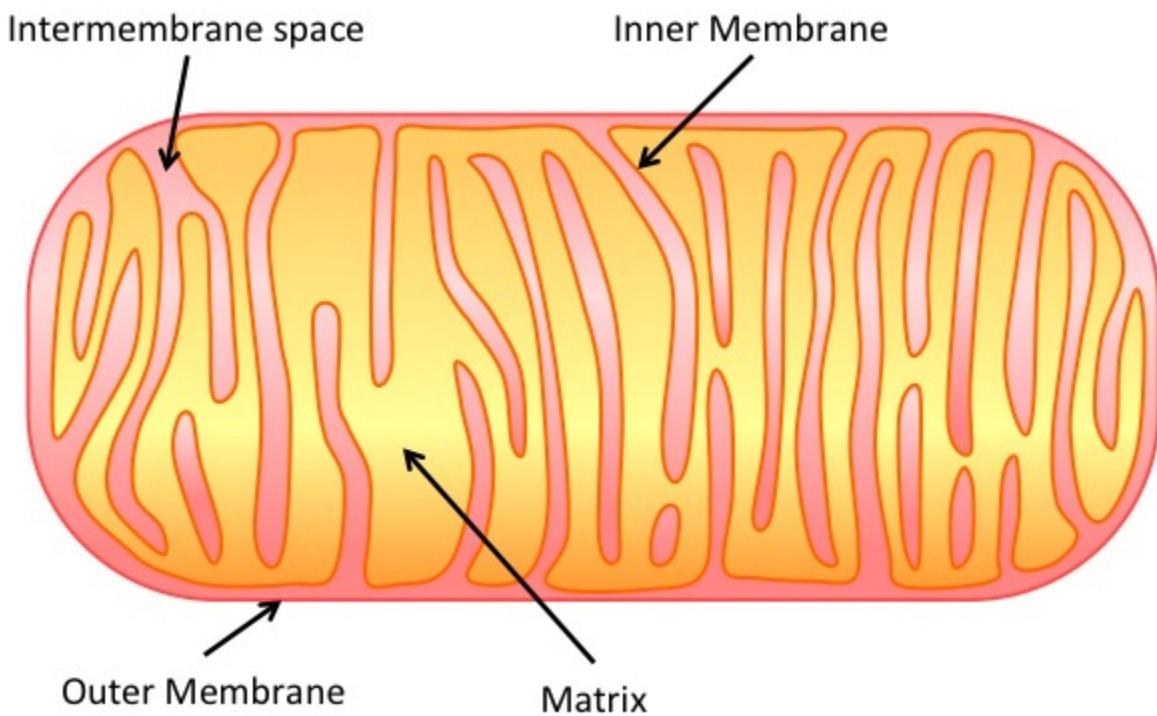
Acetyl-CoA enters into the **Krebs cycle**, a series of mitochondrial reactions that completes the breakdown of the original glucose, thereby releasing CO_2 . In this third stage of the process, energy is harvested in the form of high-energy electrons being used to generate NADH as well as another high-energy electron carrier, FADH_2 . The reactions of the Krebs cycle also produce a small amount of ATP.

So far, a minimal amount of ATP has been produced, but a lot of energy has been stored in the electron carriers NADH and FADH_2 . In the final stage of aerobic cellular respiration, **Oxidative Phosphorylation**, a series of enzymes known as the **electron transport chain** uses those high-energy electrons to produce a large amount of ATP. The high energy electrons harvested in the first three stages, and ferried by electron carriers (NADH and FADH_2) to the electron transport chain, are used to produce large amounts of ATP via the mitochondrial membrane protein known as the **ATP synthase**. During this final stage is also when atmospheric oxygen is used as the final electron and hydrogen ion acceptor, in a reaction which produces water. The need for O_2 in this final step means that these reactions are part of aerobic cellular respiration.

Location and Structures of Aerobic Cellular Respiration

All eukaryotic cells (protists, fungi, plants and animals) have mitochondria, and mitochondria are often called the power plants of the cell because these organelles produce a large amount of ATP. As you may remember from a previous module, the mitochondrion is an organelle that is hypothesized to have originated as an endosymbiotic aerobic bacteria. Some of the evidence for this hypothesis comes from the relationship of the functional parts of the mitochondria ([\[link\]](#)) to the structure of a typical aerobic bacteria. There is

an **outer membrane** which defines the organelle and represents the membrane which enveloped the bacteria when it was taken into the cell via endocytosis. The inner membrane represents the plasma membrane of the bacteria; the inner and outer membranes together form the **intermembrane space**. The **inner membrane** is highly folded; these folds are called **cristae**. The extensive folding increases the surface area for the numerous electron transport chain enzymes and the ATP synthases that are used to make ATP. In bacteria all of these enzymes are packed into the plasma membrane, as one would expect if the endosymbiotic hypothesis is correct. The production of ATP is driven by a concentration gradient between the outer and inner compartment; in aerobic bacteria this concentration gradient is between the inside and the outside of the cell. The innermost compartment, derived from the cytoplasm of the ancestral bacteria, is called the **matrix**, and this compartment (just like the cytoplasm of today's bacteria) contains ribosomes and DNA; It is also the location of the Krebs Cycle reactions.



This image illustrates the structures within the mitochondria. (Image

by Eva Horne and Robert Bear)

Fermentation

LEARNING OBJECTIVES

- Define fermentation and explain why it does not require oxygen
- Describe the fermentation pathways and their end products and give examples of microorganisms that use these pathways
- Compare and contrast fermentation and anaerobic respiration

Many cells are unable to carry out respiration because of one or more of the following circumstances:

1. The cell lacks a sufficient amount of any appropriate, inorganic, final electron acceptor to carry out cellular respiration.
2. The cell lacks genes to make appropriate complexes and electron carriers in the electron transport system.
3. The cell lacks genes to make one or more enzymes in the Krebs cycle.

Whereas lack of an appropriate inorganic final electron acceptor is environmentally dependent, the other two conditions are genetically determined. Thus, many prokaryotes, including members of the clinically important genus *Streptococcus*, are permanently incapable of respiration, even in the presence of oxygen. Conversely, many prokaryotes are facultative, meaning that, should the environmental conditions change to provide an appropriate inorganic final electron acceptor for respiration, organisms containing all the genes required to do so will switch to cellular respiration for glucose metabolism because respiration allows for much greater ATP production per glucose molecule.

If respiration does not occur, NADH must be reoxidized to NAD⁺ for reuse as an electron carrier for glycolysis, the cell's only mechanism for producing any ATP, to continue. Some living systems use an organic molecule (commonly pyruvate) as a final electron acceptor through a process called **fermentation**. Fermentation does not involve an electron transport system and does not directly produce any additional ATP beyond that produced during glycolysis by substrate-level phosphorylation. Organisms carrying out fermentation, called fermenters, produce a maximum of two ATP molecules per glucose during glycolysis. [\[link\]](#) compares the final electron acceptors and methods of ATP synthesis in aerobic respiration, anaerobic respiration, and fermentation. Note that the number of ATP molecules shown for glycolysis assumes the Embden-Meyerhof-Parnas pathway. The number of ATP molecules made by substrate-level phosphorylation (SLP) versus oxidative phosphorylation (OP) are indicated.

Comparison of Respiration Versus Fermentation

Type of Metabolism	Example	Final Electron Acceptor	Pathways Involved in ATP Synthesis (Type of Phosphorylation)	Maximum Yield of ATP Molecules

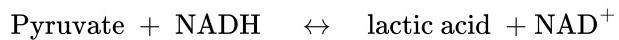
Comparison of Respiration Versus Fermentation

Type of Metabolism	Example	Final Electron Acceptor	Pathways Involved in ATP Synthesis (Type of Phosphorylation)	Maximum Yield of ATP Molecules
Aerobic respiration	<i>Pseudomonas aeruginosa</i>	O_2	EMP glycolysis (SLP) Krebs cycle (SLP) Electron transport and chemiosmosis (OP):	2 2 34
			Total	38
Anaerobic respiration	<i>Paracoccus denitrificans</i>	NO_3^- , SO_4^{2-} , Fe^{+3} , CO_2 , other inorganics	EMP glycolysis (SLP) Krebs cycle (SLP) Electron transport and chemiosmosis (OP):	2 2 1–32
			Total	5–36
Fermentation	<i>Candida albicans</i>	Organics (usually pyruvate)	EMP glycolysis (SLP) Fermentation	2 0
			Total	2

Microbial fermentation processes have been manipulated by humans and are used extensively in the production of various foods and other commercial products, including pharmaceuticals. Microbial fermentation can also be useful for identifying microbes for diagnostic purposes.

Fermentation by some bacteria, like those in yogurt and other soured food products, and by animals in muscles during oxygen depletion, is lactic acid fermentation. The chemical reaction of lactic acid fermentation is as follows:

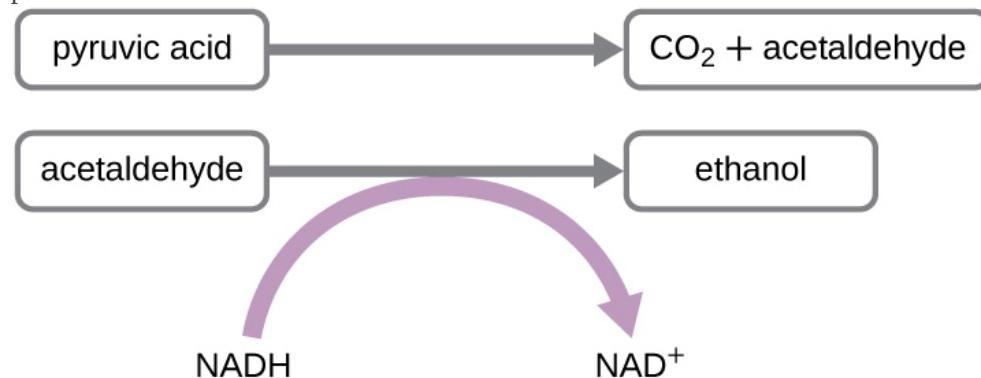
Equation:



Bacteria of several gram-positive genera, including *Lactobacillus*, *Leuconostoc*, and *Streptococcus*, are collectively known as the lactic acid bacteria (LAB), and various strains are important in food production. During yogurt and cheese production, the highly acidic environment generated by lactic acid fermentation denatures proteins contained in milk, causing it to solidify. When lactic acid is the only fermentation product, the process is said to be **homolactic fermentation**; such is the case for *Lactobacillus delbrueckii* and *S. thermophilus* used in yogurt production. However, many bacteria perform **heterolactic fermentation**, producing a mixture of lactic acid, ethanol and/or acetic acid, and CO_2 as a result, because of their use of the branched pentose phosphate pathway instead of the EMP pathway for glycolysis. One important heterolactic fermenter is *Leuconostoc mesenteroides*, which is used for souring vegetables like cucumbers and cabbage, producing pickles and sauerkraut, respectively.

Lactic acid bacteria are also important medically. The production of low pH environments within the body inhibits the establishment and growth of pathogens in these areas. For example, the vaginal microbiota is composed largely of lactic acid bacteria, but when these bacteria are reduced, yeast can proliferate, causing a yeast infection. Additionally, lactic acid bacteria are important in maintaining the health of the gastrointestinal tract and, as such, are the primary component of probiotics.

Another familiar fermentation process is alcohol fermentation, which produces ethanol. The ethanol fermentation reaction is shown in [\[link\]](#). In the first reaction, the enzyme pyruvate decarboxylase removes a carboxyl group from pyruvate, releasing CO₂ gas while producing the two-carbon molecule acetaldehyde. The second reaction, catalyzed by the enzyme alcohol dehydrogenase, transfers an electron from NADH to acetaldehyde, producing ethanol and NAD⁺. The ethanol fermentation of pyruvate by the yeast *Saccharomyces cerevisiae* is used in the production of alcoholic beverages and also makes bread products rise due to CO₂ production. Outside of the food industry, ethanol fermentation of plant products is important in biofuel production.



The chemical reactions of alcohol fermentation are shown here. Ethanol fermentation is important in the production of alcoholic beverages and bread.

Beyond lactic acid fermentation and alcohol fermentation, many other fermentation methods occur in prokaryotes, all for the purpose of ensuring an adequate supply of NAD⁺ for glycolysis ([\[link\]](#)). Without these pathways, glycolysis would not occur and no ATP would be harvested from the breakdown of glucose. It should be noted that most forms of fermentation besides homolactic fermentation produce gas, commonly CO₂ and/or hydrogen gas. Many of these different types of fermentation pathways are also used in food production and each results in the production of different organic acids, contributing to the unique flavor of a particular fermented food product. The propionic acid produced during propionic acid fermentation contributes to the distinctive flavor of Swiss cheese, for example.

Several fermentation products are important commercially outside of the food industry. For example, chemical solvents such as acetone and butanol are produced during acetone-butanol-ethanol fermentation. Complex organic pharmaceutical compounds used in antibiotics (e.g., penicillin), vaccines, and vitamins are produced through mixed acid fermentation. Fermentation products are used in the laboratory to differentiate various bacteria for diagnostic purposes. For example, enteric bacteria are known for their ability to perform mixed acid fermentation, reducing the pH, which can be detected using a pH indicator. Similarly, the bacterial production of acetoin during butanediol fermentation can also be detected. Gas production from fermentation can also be seen in an inverted Durham tube that traps produced gas in a broth culture.

Microbes can also be differentiated according to the substrates they can ferment. For example, *E. coli* can ferment lactose, forming gas, whereas some of its close gram-negative relatives cannot. The ability to ferment the sugar alcohol sorbitol is used to identify the pathogenic enterohemorrhagic O157:H7 strain of *E. coli* because, unlike other *E. coli* strains, it is unable to ferment sorbitol. Last, mannitol fermentation differentiates the mannitol-fermenting *Staphylococcus aureus* from other non-mannitol-fermenting staphylococci.

Common Fermentation Pathways			
Pathway	End Products	Example Microbes	Commercial Products
Acetone-butanol-ethanol	Acetone, butanol, ethanol, CO ₂	<i>Clostridium acetobutylicum</i>	Commercial solvents, gasoline alternative
Alcohol	Ethanol, CO ₂	<i>Candida, Saccharomyces</i>	Beer, bread
Butanediol	Formic and lactic acid; ethanol; acetoin; 2,3 butanediol; CO ₂ ; hydrogen gas	<i>Klebsiella, Enterobacter</i>	Chardonnay wine
Butyric acid	Butyric acid, CO ₂ , hydrogen gas	<i>Clostridium butyricum</i>	Butter
Lactic acid	Lactic acid	<i>Streptococcus, Lactobacillus</i>	Sauerkraut, yogurt, cheese
Mixed acid	Acetic, formic, lactic, and succinic acids; ethanol, CO ₂ , hydrogen gas	<i>Escherichia, Shigella</i>	Vinegar, cosmetics, pharmaceuticals
Propionic acid	Acetic acid, propionic acid, CO ₂	<i>Propionibacterium, Bifidobacterium</i>	Swiss cheese

Note:

- When would a metabolically versatile microbe perform fermentation rather than cellular respiration?

Note:

Identifying Bacteria by Using API Test Panels

Identification of a microbial isolate is essential for the proper diagnosis and appropriate treatment of patients. Scientists have developed techniques that identify bacteria according to their biochemical characteristics. Typically, they either examine the use of specific carbon sources as substrates for fermentation or other metabolic reactions, or they identify fermentation products or specific enzymes present in reactions. In the past, microbiologists have used individual test tubes and plates to conduct biochemical testing. However, scientists, especially those in clinical laboratories, now more frequently use plastic, disposable, multitest panels that contain a number of miniature reaction tubes, each typically including a specific substrate and pH indicator. After inoculation of the test panel with a small sample of the microbe in question and incubation, scientists can compare the results to a database that includes the expected results for specific biochemical reactions for known microbes, thus enabling rapid identification of a sample microbe. These test panels have allowed scientists to reduce costs while improving efficiency and reproducibility by performing a larger number of tests simultaneously.

Many commercial, miniaturized biochemical test panels cover a number of clinically important groups of bacteria and yeasts. One of the earliest and most popular test panels is the Analytical Profile Index (API) panel

invented in the 1970s. Once some basic laboratory characterization of a given strain has been performed, such as determining the strain's Gram morphology, an appropriate test strip that contains 10 to 20 different biochemical tests for differentiating strains within that microbial group can be used. Currently, the various API strips can be used to quickly and easily identify more than 600 species of bacteria, both aerobic and anaerobic, and approximately 100 different types of yeasts. Based on the colors of the reactions when metabolic end products are present, due to the presence of pH indicators, a metabolic profile is created from the results ([\[link\]](#)). Microbiologists can then compare the sample's profile to the database to identify the specific microbe.



The API 20NE test strip is used to identify specific strains of gram-negative bacteria outside the Enterobacteriaceae. Here is an API 20NE test strip result for *Photobacterium damsela ssp. piscicida*.

Note:

Part 2

Many of Hannah's symptoms are consistent with several different infections, including influenza and pneumonia. However, her sluggish reflexes along with her light sensitivity and stiff neck suggest some possible involvement of the central nervous system, perhaps indicating meningitis. Meningitis is an infection of the cerebrospinal fluid (CSF) around the brain and spinal cord that causes inflammation of the meninges, the protective layers covering the brain. Meningitis can be caused by viruses, bacteria, or fungi. Although all forms of meningitis are serious, bacterial meningitis is particularly serious. Bacterial meningitis may be caused by several different bacteria, but the bacterium *Neisseria meningitidis*, a gram-negative, bean-shaped diplococcus, is a common cause and leads to death within 1 to 2 days in 5% to 10% of patients.

Given the potential seriousness of Hannah's conditions, her physician advised her parents to take her to the hospital in the Gambian capital of Banjul and there have her tested and treated for possible meningitis. After a 3-hour drive to the hospital, Hannah was immediately admitted. Physicians took a blood sample and performed a lumbar puncture to test her CSF. They also immediately started her on a course of the antibiotic ceftriaxone, the drug of choice for treatment of meningitis caused by *N. meningitidis*, without waiting for laboratory test results.

- How might biochemical testing be used to confirm the identity of *N. meningitidis*?
- Why did Hannah's doctors decide to administer antibiotics without waiting for the test results?

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

Key Concepts and Summary

- Fermentation uses an organic molecule as a final electron acceptor to regenerate NAD⁺ from NADH so that glycolysis can continue.
- Fermentation does not involve an electron transport system, and no ATP is made by the fermentation process directly. Fermenters make very little ATP—only two ATP molecules per glucose molecule during glycolysis.
- Microbial fermentation processes have been used for the production of foods and pharmaceuticals, and for the identification of microbes.
- During lactic acid fermentation, pyruvate accepts electrons from NADH and is reduced to lactic acid. Microbes performing **homolactic fermentation** produce only lactic acid as the fermentation product;

microbes performing **heterolactic fermentation** produce a mixture of lactic acid, ethanol and/or acetic acid, and CO₂.

- Lactic acid production by the normal microbiota prevents growth of pathogens in certain body regions and is important for the health of the gastrointestinal tract.
- During ethanol fermentation, pyruvate is first decarboxylated (releasing CO₂) to acetaldehyde, which then accepts electrons from NADH, reducing acetaldehyde to ethanol. Ethanol fermentation is used for the production of alcoholic beverages, for making bread products rise, and for biofuel production.
- Fermentation products of pathways (e.g., propionic acid fermentation) provide distinctive flavors to food products. Fermentation is used to produce chemical solvents (acetone-butanol-ethanol fermentation) and pharmaceuticals (mixed acid fermentation).
- Specific types of microbes may be distinguished by their fermentation pathways and products. Microbes may also be differentiated according to the substrates they are able to ferment.

Multiple Choice

Exercise:

Problem: Which of the following is the purpose of fermentation?

- A. to make ATP
- B. to make carbon molecule intermediates for anabolism
- C. to make NADH
- D. to make NAD⁺

Solution:

D

Exercise:

Problem: Which molecule typically serves as the final electron acceptor during fermentation?

- A. oxygen
- B. NAD⁺
- C. pyruvate
- D. CO₂

Solution:

C

Exercise:

Problem: Which fermentation product is important for making bread rise?

- A. ethanol
- B. CO₂
- C. lactic acid
- D. hydrogen gas

Solution:

B

Exercise:

Problem: Which of the following is not a commercially important fermentation product?

- A. ethanol
 - B. pyruvate
 - C. butanol
 - D. penicillin
-

Solution:

B

Fill in the Blank

Exercise:

Problem:

The microbe responsible for ethanol fermentation for the purpose of producing alcoholic beverages is _____.

Solution:

yeast (*Saccharomyces cerevisiae*)

Exercise:

Problem:

_____ results in the production of a mixture of fermentation products, including lactic acid, ethanol and/or acetic acid, and CO₂.

Solution:

Heterolactic fermentation

Exercise:

Problem: Fermenting organisms make ATP through the process of _____.

Solution:

glycolysis

Matching

Exercise:

Problem: Match the fermentation pathway with the correct commercial product it is used to produce:

<input type="checkbox"/> acetone-butanol-ethanol fermentation	a. bread
<input type="checkbox"/> alcohol fermentation	b. pharmaceuticals
<input type="checkbox"/> lactic acid fermentation	c. Swiss cheese
<input type="checkbox"/> mixed acid fermentation	d. yogurt
<input type="checkbox"/> propionic acid fermentation	e. industrial solvents

Solution:

e; 2. a; 3. d; 4. b; 5. c

Short Answer

Exercise:

Problem:

Why are some microbes, including *Streptococcus* spp., unable to perform aerobic respiration, even in the presence of oxygen?

Exercise:

Problem: How can fermentation be used to differentiate various types of microbes?

Critical Thinking

Exercise:

Problem:

The bacterium *E. coli* is capable of performing aerobic respiration, anaerobic respiration, and fermentation. When would it perform each process and why? How is ATP made in each case?

Catabolism of Lipids and Proteins

LEARNING OBJECTIVES

- Describe how lipids are catabolized
- Describe how lipid catabolism can be used to identify microbes
- Describe how proteins are catabolized
- Describe how protein catabolism can be used to identify bacteria

Previous sections have discussed the catabolism of glucose, which provides energy to living cells, as well as how polysaccharides like glycogen, starch, and cellulose are degraded to glucose monomers. But microbes consume more than just carbohydrates for food. In fact, the microbial world is known for its ability to degrade a wide range of molecules, both naturally occurring and those made by human processes, for use as carbon sources. In this section, we will see that the pathways for both lipid and protein catabolism connect to those used for carbohydrate catabolism, eventually leading into glycolysis, the transition reaction, and the Krebs cycle pathways. Metabolic pathways should be considered to be porous—that is, substances enter from other pathways, and intermediates leave for other pathways. These pathways are not closed systems. Many of the substrates, intermediates, and products in a particular pathway are reactants in other pathways.

Lipid Catabolism

Triglycerides are a form of long-term energy storage in animals. They are made of glycerol and three fatty acids (see [[link](#)]). Phospholipids compose

the cell and organelle membranes of all organisms except the archaea. Phospholipid structure is similar to triglycerides except that one of the fatty acids is replaced by a phosphorylated head group (see [[link](#)]). Triglycerides and phospholipids are broken down first by releasing fatty acid chains (and/or the phosphorylated head group, in the case of phospholipids) from the three-carbon glycerol backbone. The reactions breaking down triglycerides are catalyzed by **lipases** and those involving phospholipids are catalyzed by **phospholipases**. These enzymes contribute to the virulence of certain microbes, such as the bacterium *Staphylococcus aureus* and the fungus *Cryptococcus neoformans*. These microbes use phospholipases to destroy lipids and phospholipids in host cells and then use the catabolic products for energy (see [Virulence Factors of Bacterial and Viral Pathogens](#)).

The resulting products of lipid catabolism, glycerol and fatty acids, can be further degraded. Glycerol can be phosphorylated to glycerol-3-phosphate and easily converted to glyceraldehyde 3-phosphate, which continues through glycolysis. The released fatty acids are catabolized in a process called **β -oxidation**, which sequentially removes two-carbon acetyl groups from the ends of fatty acid chains, reducing NAD⁺ and FAD to produce NADH and FADH₂, respectively, whose electrons can be used to make ATP by oxidative phosphorylation. The acetyl groups produced during β -oxidation are carried by coenzyme A to the Krebs cycle, and their movement through this cycle results in their degradation to CO₂, producing ATP by substrate-level phosphorylation and additional NADH and FADH₂ molecules (see [Appendix C](#) for a detailed illustration of β -oxidation).

Other types of lipids can also be degraded by certain microbes. For example, the ability of certain pathogens, like *Mycobacterium tuberculosis*, to degrade cholesterol contributes to their virulence. The side chains of cholesterol can be easily removed enzymatically, but degradation of the remaining fused rings is more problematic. The four fused rings are sequentially broken in a multistep process facilitated by specific enzymes, and the resulting products, including pyruvate, can be further catabolized in the Krebs cycle.

Note:

- How can lipases and phospholipases contribute to virulence in microbes?

Protein Catabolism

Proteins are degraded through the concerted action of a variety of microbial **protease** enzymes. Extracellular proteases cut proteins internally at specific amino acid sequences, breaking them down into smaller peptides that can then be taken up by cells. Some clinically important pathogens can be identified by their ability to produce a specific type of extracellular protease. For example, the production of the extracellular protease gelatinase by members of the genera *Proteus* and *Serratia* can be used to distinguish them from other gram-negative enteric bacteria. Following inoculation and growth of microbes in gelatin broth, degradation of the gelatin protein due to gelatinase production prevents solidification of gelatin when refrigerated. Other pathogens can be distinguished by their ability to degrade casein, the main protein found in milk. When grown on skim milk agar, production of the extracellular protease caseinase causes degradation of casein, which appears as a zone of clearing around the microbial growth. Caseinase production by the opportunist pathogen *Pseudomonas aeruginosa* can be used to distinguish it from other related gram-negative bacteria.

After extracellular protease degradation and uptake of peptides in the cell, the peptides can then be broken down further into individual amino acids by additional intracellular proteases, and each amino acid can be enzymatically deaminated to remove the amino group. The remaining molecules can then enter the transition reaction or the Krebs cycle.

Note:

- How can protein catabolism help identify microbes?

Note:

Part 3

Because bacterial meningitis progresses so rapidly, Hannah's doctors had decided to treat her aggressively with antibiotics, based on empirical observation of her symptoms. However, laboratory testing to confirm the cause of Hannah's meningitis was still important for several reasons. *N. meningitidis* is an infectious pathogen that can be spread from person to person through close contact; therefore, if tests confirm *N. meningitidis* as the cause of Hannah's symptoms, Hannah's parents and others who came into close contact with her might need to be vaccinated or receive prophylactic antibiotics to lower their risk of contracting the disease. On the other hand, if it turns out that *N. meningitidis* is not the cause, Hannah's doctors might need to change her treatment.

The clinical laboratory performed a Gram stain on Hannah's blood and CSF samples. The Gram stain showed the presence of a bean-shaped gram-negative diplococcus. The technician in the hospital lab cultured Hannah's blood sample on both blood agar and chocolate agar, and the bacterium that grew on both media formed gray, nonhemolytic colonies. Next, he performed an oxidase test on this bacterium and determined that it was oxidase positive. Last, he examined the repertoire of sugars that the bacterium could use as a carbon source and found that the bacterium was positive for glucose and maltose use but negative for lactose and sucrose use. All of these test results are consistent with characteristics of *N. meningitidis*.

- What do these test results tell us about the metabolic pathways of *N. meningitidis*?
- Why do you think that the hospital used these biochemical tests for identification in lieu of molecular analysis by DNA testing?

Jump to the [next Clinical Focus box](#). Go back to the [previous Clinical Focus box](#).

Key Concepts and Summary

- Collectively, microbes have the ability to degrade a wide variety of carbon sources besides carbohydrates, including lipids and proteins. The catabolic pathways for all of these molecules eventually connect into glycolysis and the Krebs cycle.
- Several types of lipids can be microbially degraded. Triglycerides are degraded by extracellular **lipases**, releasing fatty acids from the glycerol backbone. Phospholipids are degraded by **phospholipases**, releasing fatty acids and the phosphorylated head group from the glycerol backbone. Lipases and phospholipases act as virulence factors for certain pathogenic microbes.
- Fatty acids can be further degraded inside the cell through **β -oxidation**, which sequentially removes two-carbon acetyl groups from the ends of fatty acid chains.
- Protein degradation involves extracellular **proteases** that degrade large proteins into smaller peptides. Detection of the extracellular proteases gelatinase and caseinase can be used to differentiate clinically relevant bacteria.

Multiple Choice

Exercise:

Problem:

Which of the following molecules is not produced during the breakdown of phospholipids?

- A. glucose
- B. glycerol
- C. acetyl groups
- D. fatty acids

Solution:

A

Exercise:

Problem: Caseinase is which type of enzyme?

- A. phospholipase
 - B. lipase
 - C. extracellular protease
 - D. intracellular protease
-

Solution:

C

Exercise:

Problem:

Which of the following is the first step in triglyceride degradation?

- A. removal of fatty acids
 - B. β -oxidation
 - C. breakage of fused rings
 - D. formation of smaller peptides
-

Solution:

A

Fill in the Blank

Exercise:

Problem:

The process by which two-carbon units are sequentially removed from fatty acids, producing acetyl-CoA, FADH₂, and NADH is called _____.

Solution:

β -oxidation

Exercise:

Problem:

The NADH and FADH₂ produced during β -oxidation are used to make

_____.

Solution:

ATP by oxidative phosphorylation

Exercise:

Problem:

_____ is a type of medium used to detect the production of an extracellular protease called caseinase.

Solution:

Skim milk agar

Short Answer

Exercise:

Problem:

How are the products of lipid and protein degradation connected to glucose metabolism pathways?

Exercise:

Problem:

What is the general strategy used by microbes for the degradation of macromolecules?

Critical Thinking

Exercise:

Problem:

Do you think that β -oxidation can occur in an organism incapable of cellular respiration? Why or why not?

Photosynthesis

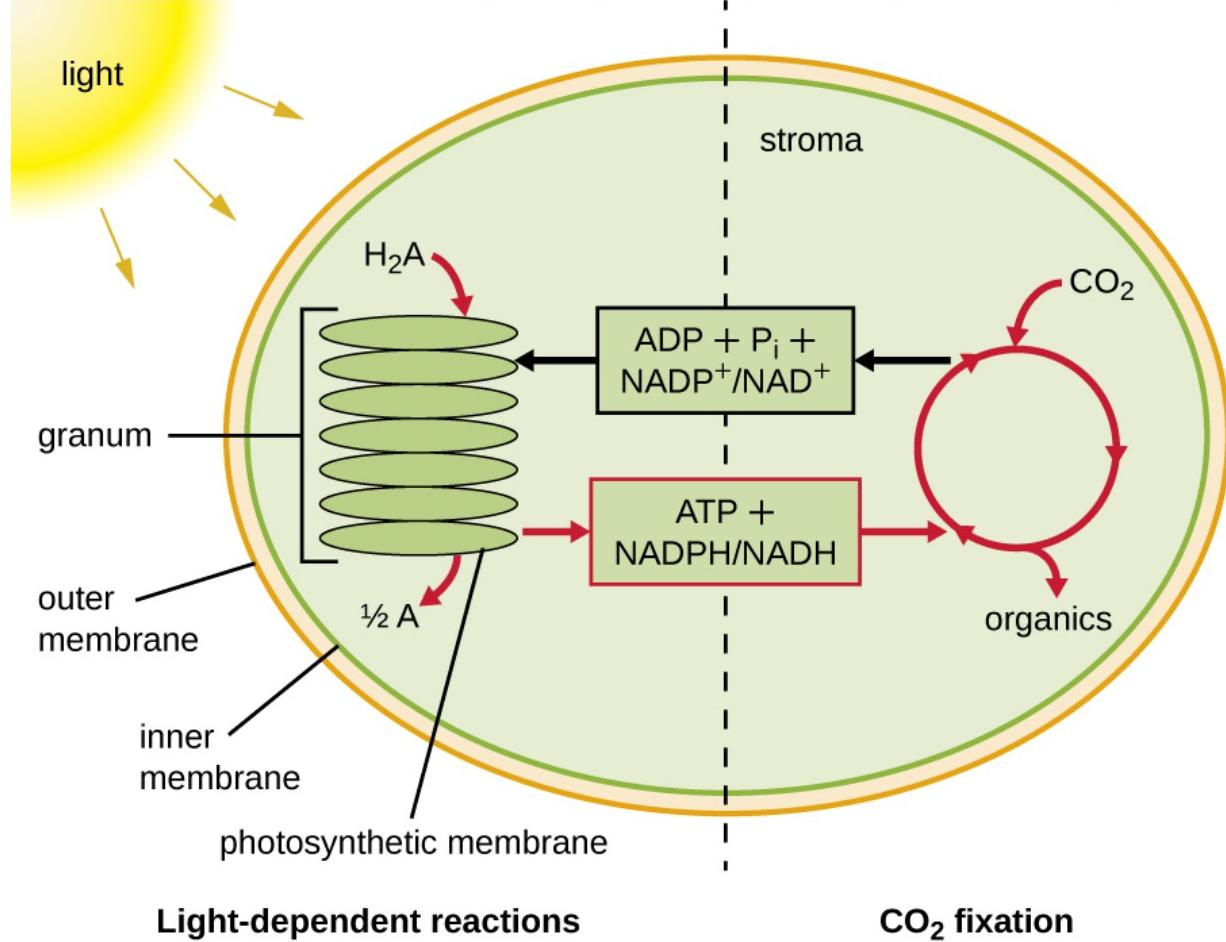
LEARNING OBJECTIVES

- Describe the function and locations of photosynthetic pigments in eukaryotes and prokaryotes
- Describe the major products of the light-dependent and light-independent reactions
- Describe the reactions that produce glucose in a photosynthetic cell
- Compare and contrast cyclic and noncyclic photophosphorylation

Heterotrophic organisms ranging from *E. coli* to humans rely on the chemical energy found mainly in carbohydrate molecules. Many of these carbohydrates are produced by **photosynthesis**, the biochemical process by which phototrophic organisms convert solar energy (sunlight) into chemical energy. Although photosynthesis is most commonly associated with plants, microbial photosynthesis is also a significant supplier of chemical energy, fueling many diverse ecosystems. In this section, we will focus on microbial photosynthesis.

Photosynthesis takes place in two sequential stages: the light-dependent reactions and the light-independent reactions ([\[link\]](#)). In the **light-dependent reactions**, energy from sunlight is absorbed by pigment molecules in photosynthetic membranes and converted into stored chemical energy. In the **light-independent reactions**, the chemical energy produced by the light-dependent reactions is used to drive the assembly of sugar molecules using CO₂; however, these reactions are still light dependent

because the products of the light-dependent reactions necessary for driving them are short-lived. The light-dependent reactions produce ATP and either NADPH or NADH to temporarily store energy. These energy carriers are used in the light-independent reactions to drive the energetically unfavorable process of “fixing” inorganic CO₂ in an organic form, sugar.



The light-dependent reactions of photosynthesis (left) convert light energy into chemical energy, forming ATP and NADPH. These products are used by the light-independent reactions to fix CO₂, producing organic carbon molecules.

Photosynthetic Structures in Eukaryotes and Prokaryotes

In all phototrophic eukaryotes, photosynthesis takes place inside a **chloroplast**, an organelle that arose in eukaryotes by endosymbiosis of a photosynthetic bacterium (see [Unique Characteristics of Eukaryotic Cells](#)). These chloroplasts are enclosed by a double membrane with inner and outer layers. Within the chloroplast is a third membrane that forms stacked, disc-shaped photosynthetic structures called thylakoids ([\[link\]](#)). A stack of thylakoids is called a grana, and the space surrounding the grana within the chloroplast is called stroma.

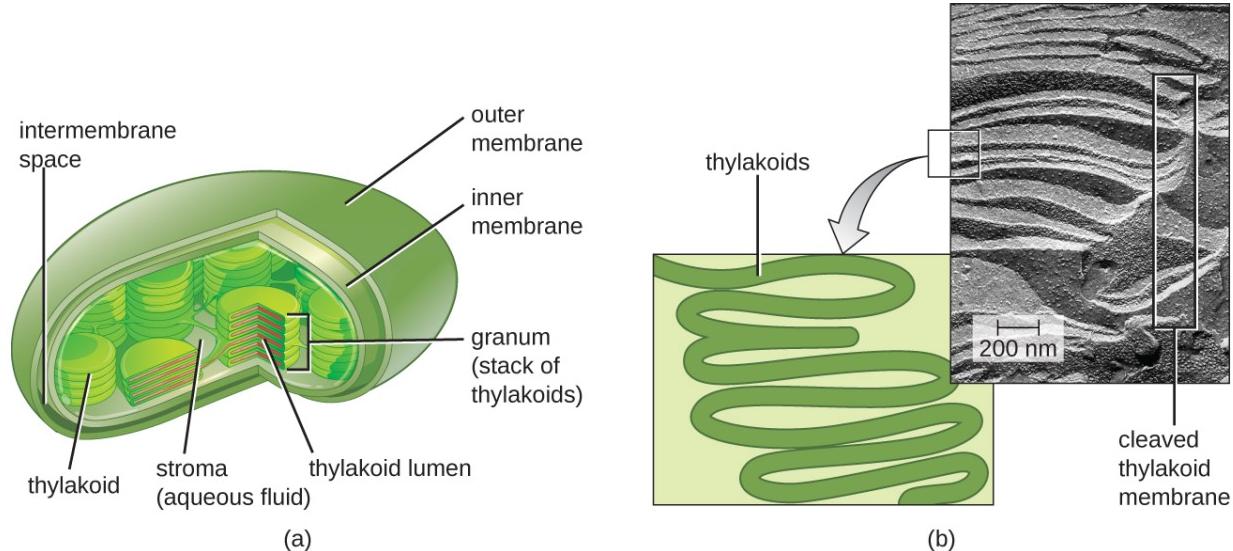
Photosynthetic membranes in prokaryotes, by contrast, are not organized into distinct membrane-enclosed organelles; rather, they are infolded regions of the plasma membrane. In cyanobacteria, for example, these infolded regions are also referred to as thylakoids. In either case, embedded within the thylakoid membranes or other photosynthetic bacterial membranes are **photosynthetic pigment** molecules organized into one or more photosystems, where light energy is actually converted into chemical energy.

Photosynthetic pigments within the photosynthetic membranes are organized into **photosystems**, each of which is composed of a light-harvesting (antennae) complex and a reaction center. The **light-harvesting complex** consists of multiple proteins and associated pigments that each may absorb light energy and, thus, become excited. This energy is transferred from one pigment molecule to another until eventually (after about a millionth of a second) it is delivered to the reaction center. Up to this point, only energy—not electrons—has been transferred between molecules. The **reaction center** contains a pigment molecule that can undergo oxidation upon excitation, actually giving up an electron. It is at this step in photosynthesis that light energy is converted into an excited electron.

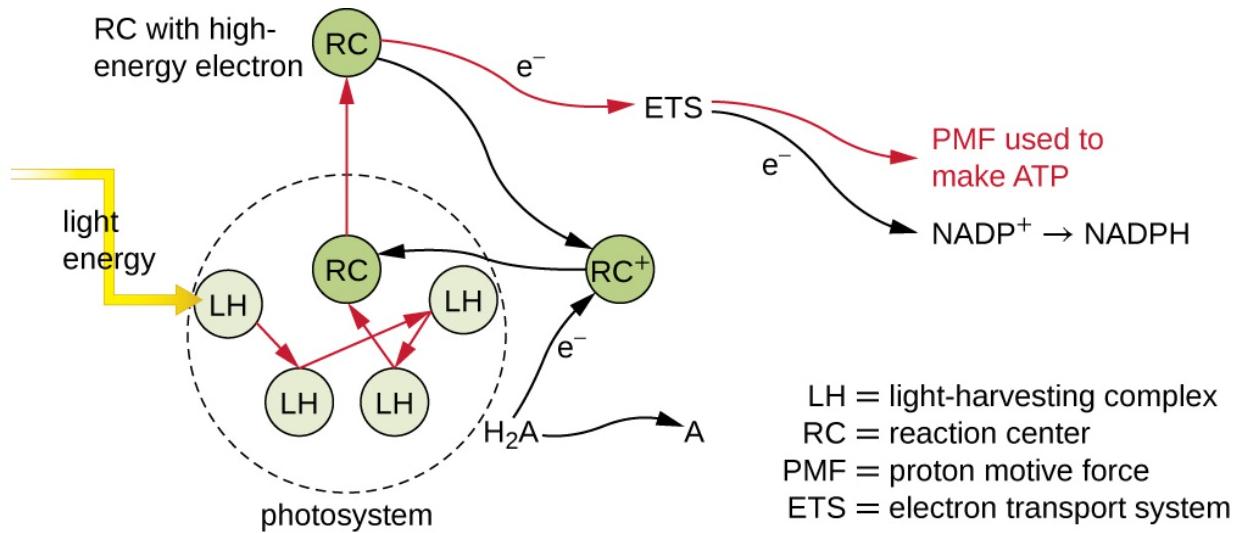
Different kinds of light-harvesting pigments absorb unique patterns of wavelengths (colors) of visible light. Pigments reflect or transmit the wavelengths they cannot absorb, making them appear the corresponding color. Examples of photosynthetic pigments (molecules used to absorb solar energy) are bacteriochlorophylls (green, purple, or red), carotenoids (orange, red, or yellow), chlorophylls (green), phycocyanins (blue), and

phycoerythrins (red). By having mixtures of pigments, an organism can absorb energy from more wavelengths. Because photosynthetic bacteria commonly grow in competition for sunlight, each type of photosynthetic bacteria is optimized for harvesting the wavelengths of light to which it is commonly exposed, leading to stratification of microbial communities in aquatic and soil ecosystems by light quality and penetration.

Once the light harvesting complex transfers the energy to the reaction center, the reaction center delivers its high-energy electrons, one by one, to an electron carrier in an electron transport system, and electron transfer through the ETS is initiated. The ETS is similar to that used in cellular respiration and is embedded within the photosynthetic membrane. Ultimately, the electron is used to produce NADH or NADPH. The electrochemical gradient that forms across the photosynthetic membrane is used to generate ATP by chemiosmosis through the process of photophosphorylation, another example of oxidative phosphorylation ([\[link\]](#)).



(a) Photosynthesis in eukaryotes takes place in chloroplasts, which contain thylakoids stacked into grana. (b) A photosynthetic prokaryote has infolded regions of the plasma membrane that function like thylakoids. (credit: scale bar data from Matt Russell.)



This figure summarizes how a photosystem works. Light harvesting (LH) pigments absorb light energy, converting it to chemical energy. The energy is passed from one LH pigment to another until it reaches a reaction center (RC) pigment, exciting an electron. This high-energy electron is lost from the RC pigment and passed through an electron transport system (ETS), ultimately producing NADH or NADPH and ATP. A reduced molecule (H_2A) donates an electron, replacing electrons to the electron-deficient RC pigment.

Note:

- In a phototrophic eukaryote, where does photosynthesis take place?

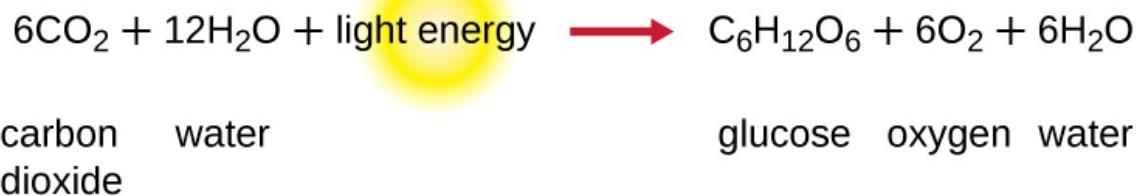
Oxygenic and Anoxygenic Photosynthesis

For photosynthesis to continue, the electron lost from the reaction center pigment must be replaced. The source of this electron (H_2A) differentiates the **oxygenic photosynthesis** of plants and cyanobacteria from **anoxygenic photosynthesis** carried out by other types of bacterial phototrophs ([\[link\]](#)). In oxygenic photosynthesis, H_2O is split and supplies the electron to the reaction center. Because oxygen is generated as a byproduct and is released, this type of photosynthesis is referred to as oxygenic photosynthesis. However, when other reduced compounds serve as the electron donor, oxygen is not generated; these types of photosynthesis are called anoxygenic photosynthesis. Hydrogen sulfide (H_2S) or thiosulfate ($S_2O_3^{2-}$) can serve as the electron donor, generating elemental sulfur and sulfate (SO_4^{2-}) ions, respectively, as a result.

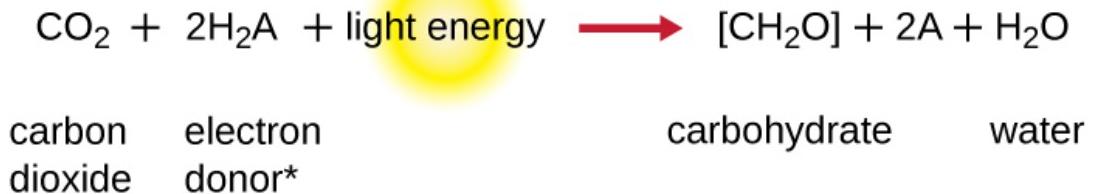
Photosystems have been classified into two types: photosystem I (PSI) and photosystem II (PSII) ([\[link\]](#)). Cyanobacteria and plant chloroplasts have both photosystems, whereas anoxygenic photosynthetic bacteria use only one of the photosystems. Both photosystems are excited by light energy simultaneously. If the cell requires both ATP and NADPH for biosynthesis, then it will carry out **noncyclic photophosphorylation**. Upon passing of the PSII reaction center electron to the ETS that connects PSII and PSI, the lost electron from the PSII reaction center is replaced by the splitting of water. The excited PSI reaction center electron is used to reduce $NADP^+$ to NADPH and is replaced by the electron exiting the ETS. The flow of electrons in this way is called the **Z-scheme**.

If a cell's need for ATP is significantly greater than its need for NADPH, it may bypass the production of reducing power through **cyclic photophosphorylation**. Only PSI is used during cyclic photophosphorylation; the high-energy electron of the PSI reaction center is passed to an ETS carrier and then ultimately returns to the oxidized PSI reaction center pigment, thereby reducing it.

Oxygenic photosynthesis

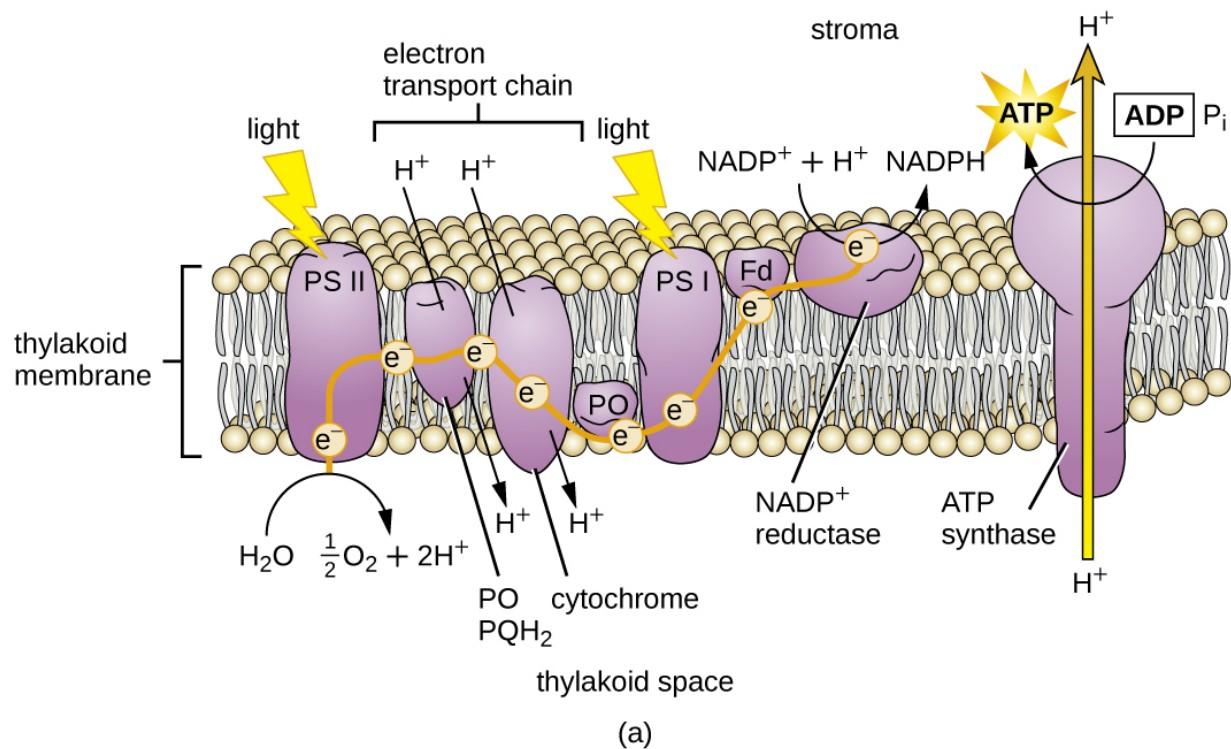


Anoxygenic photosynthesis

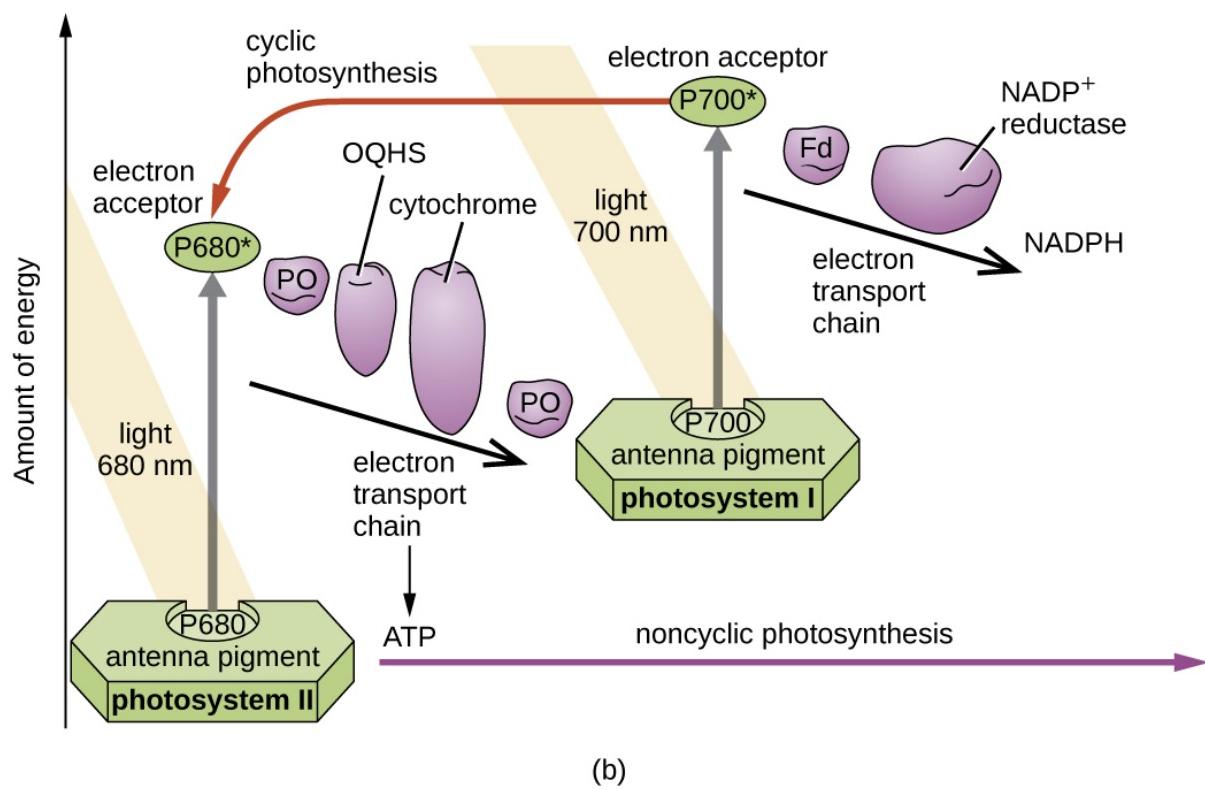


* $\text{H}_2\text{A} = \text{H}_2\text{O}, \text{H}_2\text{S}, \text{H}_2$, or other electron donor

Eukaryotes and cyanobacteria carry out oxygenic photosynthesis, producing oxygen, whereas other bacteria carry out anoxygenic photosynthesis, which does not produce oxygen.



(a)



(b)

(a) PSI and PSII are found on the thylakoid membrane. The high-

energy electron from PSII is passed to an ETS, which generates a proton motive force for ATP synthesis by chemiosmosis, and ultimately replaces the electron lost by the PSI reaction center. The PSI reaction center electron is used to make NADPH. (b) When both ATP and NADPH are required, noncyclic photophosphorylation (in cyanobacteria and plants) provides both. The electron flow described here is referred to as the Z-scheme (shown in yellow in [a]). When the cell's ATP needs outweigh those for NADPH, cyanobacteria and plants will use only PSI, and its reaction center electron is passed to the ETS to generate a proton motive force used for ATP synthesis.

Note:

- Why would a photosynthetic bacterium have different pigments?

Light-Independent Reactions

After the energy from the sun is converted into chemical energy and temporarily stored in ATP and NADPH molecules (having lifespans of millionths of a second), photoautotrophs have the fuel needed to build multicarbon carbohydrate molecules, which can survive for hundreds of millions of years, for long-term energy storage. The carbon comes from CO₂, the gas that is a waste product of cellular respiration.

The **Calvin-Benson cycle** (named for Melvin Calvin [1911–1997] and Andrew Benson [1917–2015]), the biochemical pathway used for fixation of CO₂, is located within the cytoplasm of photosynthetic bacteria and in the stroma of eukaryotic chloroplasts. The light-independent reactions of the Calvin cycle can be organized into three basic stages: fixation, reduction, and regeneration (see [Appendix C](#) for a detailed illustration of the Calvin cycle).

- **Fixation:** The enzyme **ribulose bisphosphate carboxylase (RuBisCO)** catalyzes the addition of a CO₂ to ribulose bisphosphate (RuBP). This results in the production of 3-phosphoglycerate (3-PGA).
- **Reduction:** Six molecules of both ATP and NADPH (from the light-dependent reactions) are used to convert 3-PGA into glyceraldehyde 3-phosphate (G3P). Some G3P is then used to build glucose.
- **Regeneration:** The remaining G3P not used to synthesize glucose is used to regenerate RuBP, enabling the system to continue CO₂ fixation. Three more molecules of ATP are used in these regeneration reactions.

The Calvin cycle is used extensively by plants and photoautotrophic bacteria, and the enzyme RuBisCO is said to be the most plentiful enzyme on earth, composing 30%–50% of the total soluble protein in plant chloroplasts.[\[footnote\]](#) However, besides its prevalent use in photoautotrophs, the Calvin cycle is also used by many nonphotosynthetic chemoautotrophs to fix CO₂. Additionally, other bacteria and archaea use alternative systems for CO₂ fixation. Although most bacteria using Calvin cycle alternatives are chemoautotrophic, certain green sulfur photoautotrophic bacteria have been also shown to use an alternative CO₂ fixation pathway.

A. Dhingra et al. “Enhanced Translation of a Chloroplast-Expressed *RbcS* Gene Restores Small Subunit Levels and Photosynthesis in Nuclear *RbcS* Antisense Plants.” *Proceedings of the National Academy of Sciences of the United States of America* 101 no. 16 (2004):6315–6320.

Note:

- Describe the three stages of the Calvin cycle.

Key Concepts and Summary

- Heterotrophs depend on the carbohydrates produced by autotrophs, many of which are photosynthetic, converting solar energy into chemical energy.
- Different photosynthetic organisms use different mixtures of **photosynthetic pigments**, which increase the range of the wavelengths of light an organism can absorb.
- **Photosystems** (PSI and PSII) each contain a **light-harvesting complex**, composed of multiple proteins and associated pigments that absorb light energy. The **light-dependent reactions** of photosynthesis convert solar energy into chemical energy, producing ATP and NADPH or NADH to temporarily store this energy.
- In **oxygenic photosynthesis**, H_2O serves as the electron donor to replace the reaction center electron, and oxygen is formed as a byproduct. In **anoxygenic photosynthesis**, other reduced molecules like H_2S or thiosulfate may be used as the electron donor; as such, oxygen is not formed as a byproduct.
- **Noncyclic photophosphorylation** is used in oxygenic photosynthesis when there is a need for both ATP and NADPH production. If a cell's needs for ATP outweigh its needs for NADPH, then it may carry out **cyclic photophosphorylation** instead, producing only ATP.
- The **light-independent reactions** of photosynthesis use the ATP and NADPH from the light-dependent reactions to fix CO_2 into organic sugar molecules.

Multiple Choice

Exercise:

Problem:

During the light-dependent reactions, which molecule loses an electron?

- a light-harvesting pigment molecule
- a reaction center pigment molecule
- NADPH
- 3-phosphoglycerate

Solution:

B

Exercise:

Problem:

In prokaryotes, in which direction are hydrogen ions pumped by the electron transport system of photosynthetic membranes?

- A. to the outside of the plasma membrane
 - B. to the inside (cytoplasm) of the cell
 - C. to the stroma
 - D. to the intermembrane space of the chloroplast
-

Solution:

A

Exercise:

Problem:

Which of the following does not occur during cyclic photophosphorylation in cyanobacteria?

- A. electron transport through an ETS
 - B. photosystem I use
 - C. ATP synthesis
 - D. NADPH formation
-

Solution:

D

Exercise:

Problem:

Which of the following are two products of the light-dependent reactions?

- A. glucose and NADPH
 - B. NADPH and ATP
 - C. glyceraldehyde 3-phosphate and CO₂
 - D. glucose and oxygen
-

Solution:

B

True/False**Exercise:**

Problem: Photosynthesis always results in the formation of oxygen.

Solution:

False

Fill in the Blank**Exercise:****Problem:**

The enzyme responsible for CO₂ fixation during the Calvin cycle is called _____.

Solution:

ribulose bisphosphate carboxylase (RuBisCO)

Exercise:

Problem:

The types of pigment molecules found in plants, algae, and cyanobacteria are _____ and _____.

Solution:

chlorophylls and carotenoids

Short Answer

Exercise:

Problem:

Why would an organism perform cyclic phosphorylation instead of noncyclic phosphorylation?

Exercise:

Problem:

What is the function of photosynthetic pigments in the light-harvesting complex?

Critical Thinking

Exercise:

Problem:

Is life dependent on the carbon fixation that occurs during the light-independent reactions of photosynthesis? Explain.

Biogeochemical Cycles

LEARNING OBJECTIVES

- Define and describe the importance of microorganisms in the biogeochemical cycles of carbon, nitrogen, and sulfur
- Define and give an example of bioremediation

Energy flows directionally through ecosystems, entering as sunlight for phototrophs or as inorganic molecules for chemoautotrophs. The six most common elements associated with organic molecules—carbon, hydrogen, nitrogen, oxygen, phosphorus, and sulfur—take a variety of chemical forms and may exist for long periods in the atmosphere, on land, in water, or beneath earth’s surface. Geologic processes, such as erosion, water drainage, the movement of the continental plates, and weathering, all are involved in the cycling of elements on earth. Because geology and chemistry have major roles in the study of this process, the recycling of inorganic matter between living organisms and their nonliving environment is called a **biogeochemical cycle**. Here, we will focus on the function of microorganisms in these cycles, which play roles at each step, most frequently interconverting oxidized versions of molecules with reduced ones.

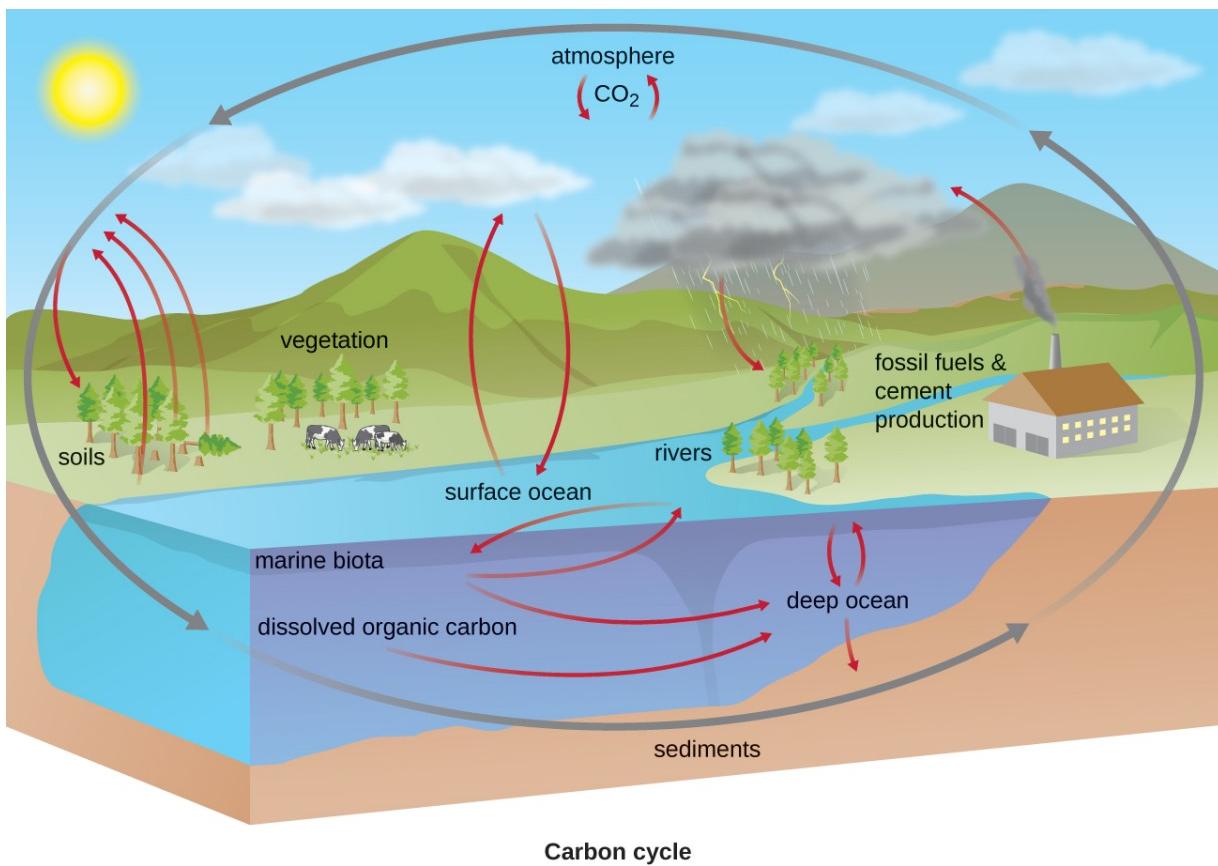
Carbon Cycle

Carbon is one of the most important elements to living organisms, as shown by its abundance and presence in all organic molecules. The carbon cycle exemplifies the connection between organisms in various ecosystems. Carbon is exchanged between heterotrophs and autotrophs within and

between ecosystems primarily by way of atmospheric CO₂, a fully oxidized version of carbon that serves as the basic building block that autotrophs use to build multicarbon, high-energy organic molecules such as glucose. Photoautotrophs and chemoautotrophs harness energy from the sun and from inorganic chemical compounds, respectively, to covalently bond carbon atoms together into reduced organic compounds whose energy can be later accessed through the processes of respiration and fermentation ([\[link\]](#)).

Overall, there is a constant exchange of CO₂ between the heterotrophs (which produce CO₂ as a result of respiration or fermentation) and the autotrophs (which use the CO₂ for fixation). Autotrophs also respire or ferment, consuming the organic molecules they form; they do not fix carbon for heterotrophs, but rather use it for their own metabolic needs.

Bacteria and archaea that use methane as their carbon source are called methanotrophs. Reduced one-carbon compounds like methane accumulate in certain anaerobic environments when CO₂ is used as a terminal electron acceptor in anaerobic respiration by archaea called methanogens. Some methanogens also ferment acetate (two carbons) to produce methane and CO₂. Methane accumulation due to methanogenesis occurs in both natural anaerobic soil and aquatic environments; methane accumulation also occurs as a result of animal husbandry because methanogens are members of the normal microbiota of ruminants. Environmental methane accumulation due to methanogenesis is of consequence because it is a strong greenhouse gas, and methanotrophs help to reduce atmospheric methane levels.



Carbon cycle

This figure summarizes the carbon cycle. Eukaryotes participate in aerobic respiration, fermentation, and oxygenic photosynthesis. Prokaryotes participate in all the steps shown. (credit: modification of work by NOAA)

Note:

- Describe the interaction between heterotrophs and autotrophs in the carbon cycle.

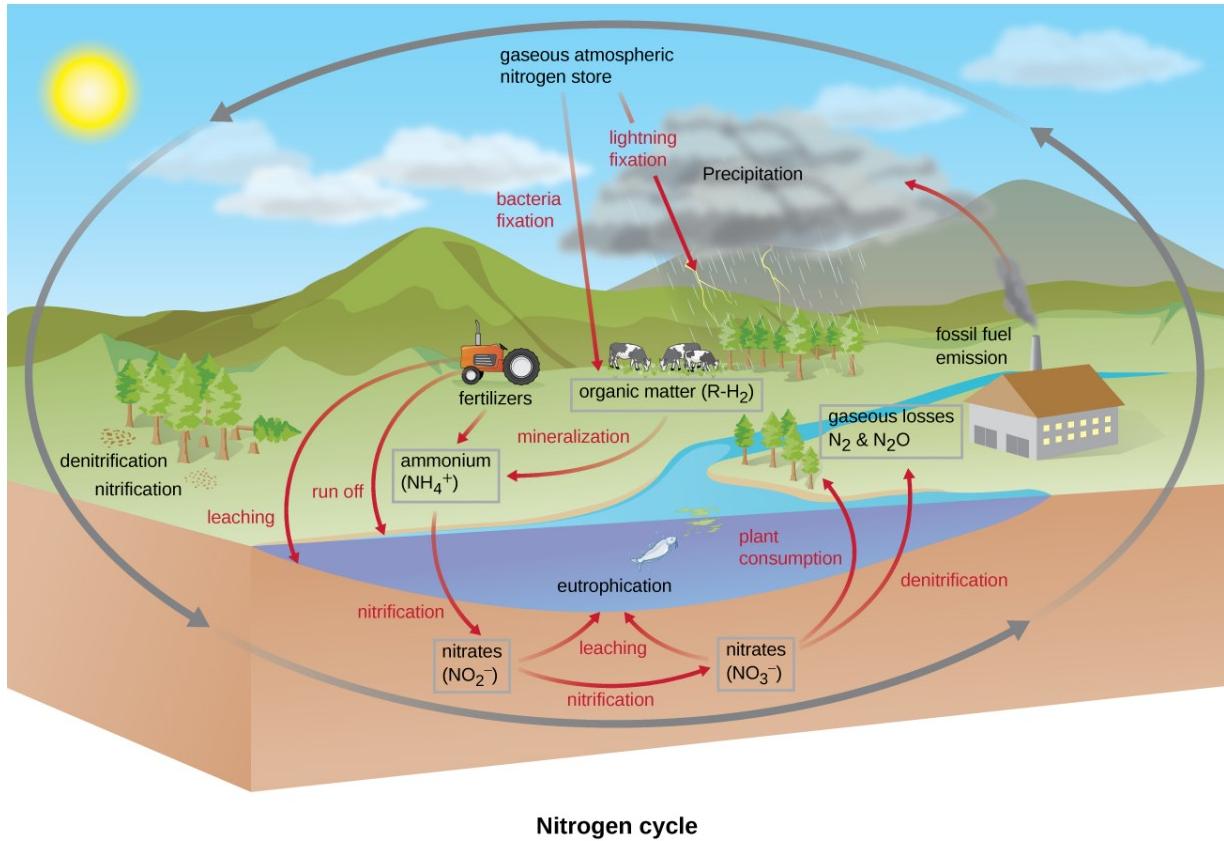
Nitrogen Cycle

Many biological macromolecules, including proteins and nucleic acids, contain nitrogen; however, getting nitrogen into living organisms is difficult. Prokaryotes play essential roles in the nitrogen cycle ([\[link\]](#)), transforming nitrogen between various forms for their own needs, benefiting other organisms indirectly. Plants and phytoplankton cannot incorporate nitrogen from the atmosphere (where it exists as tightly bonded, triple covalent N₂), even though this molecule composes approximately 78% of the atmosphere. Nitrogen enters the living world through free-living and symbiotic bacteria, which incorporate nitrogen into their macromolecules through specialized biochemical pathways called **nitrogen fixation**. Cyanobacteria in aquatic ecosystems fix inorganic nitrogen (from nitrogen gas) into ammonia (NH₃) that can be easily incorporated into biological macromolecules. *Rhizobium* bacteria ([\[link\]](#)) also fix nitrogen and live symbiotically in the root nodules of legumes (such as beans, peanuts, and peas), providing them with needed organic nitrogen while receiving fixed carbon as sugar in exchange. Free-living bacteria, such as members of the genus *Azotobacter*, are also able to fix nitrogen.

The nitrogen that enters living systems by nitrogen fixation is eventually converted from organic nitrogen back into nitrogen gas by microbes through three steps: ammonification, nitrification, and denitrification. In terrestrial systems, the first step is the ammonification process, in which certain bacteria and fungi convert nitrogenous waste from living animals or from the remains of dead organisms into ammonia (NH₃). This ammonia is then oxidized to nitrite (NO₂⁻), then to nitrate (NO₃⁻), by nitrifying soil bacteria such as members of the genus *Nitrosomonas*, through the process of nitrification. Last, the process of denitrification occurs, whereby soil bacteria, such as members of the genera *Pseudomonas* and *Clostridium*, use nitrate as a terminal electron acceptor in anaerobic respiration, converting it into nitrogen gas that reenters the atmosphere. A similar process occurs in the marine nitrogen cycle, where these three processes are performed by marine bacteria and archaea.

Human activity releases nitrogen into the environment by the use of artificial fertilizers that contain nitrogen and phosphorus compounds, which are then washed into lakes, rivers, and streams by surface runoff. A major effect from fertilizer runoff is saltwater and freshwater eutrophication, in

which nutrient runoff causes the overgrowth and subsequent death of aquatic algae, making water sources anaerobic and inhospitable for the survival of aquatic organisms.



This figure summarizes the nitrogen cycle. Note that specific groups of prokaryotes each participate in every step in the cycle. (credit: modification of work by NOAA)

Note:

- What are the three steps of the nitrogen cycle?

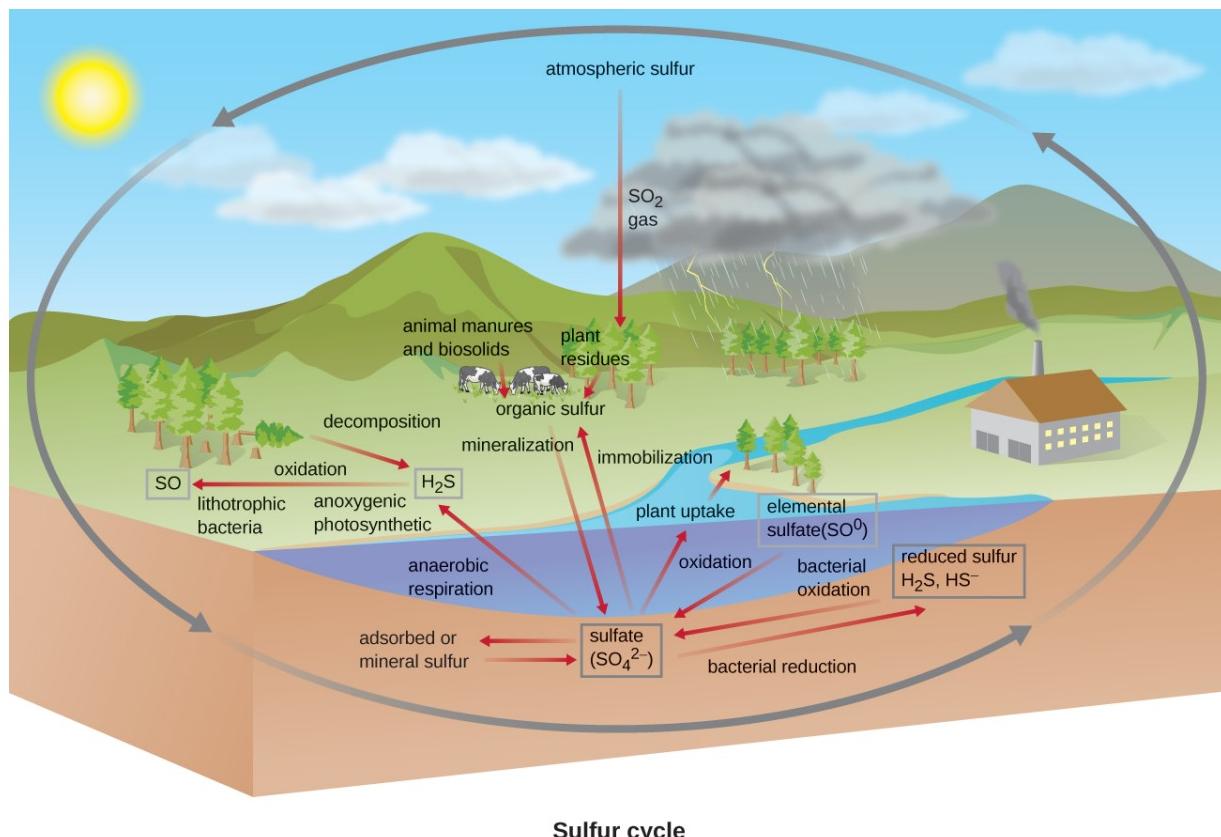
Note:

To learn more about the nitrogen cycle, visit the [PBS](#) website.

Sulfur Cycle

Sulfur is an essential element for the macromolecules of living organisms. As part of the amino acids cysteine and methionine, it is involved in the formation of proteins. It is also found in several vitamins necessary for the synthesis of important biological molecules like coenzyme A. Several groups of microbes are responsible for carrying out processes involved in the sulfur cycle ([\[link\]](#)). Anoxygenic photosynthetic bacteria as well as chemoautotrophic archaea and bacteria use hydrogen sulfide as an electron donor, oxidizing it first to elemental sulfur (S^0), then to sulfate (SO_4^{2-}). This leads to stratification of hydrogen sulfide in soil, with levels increasing at deeper, more anaerobic depths.

Many bacteria and plants can use sulfate as a sulfur source. Decomposition dead organisms by fungi and bacteria remove sulfur groups from amino acids, producing hydrogen sulfide, returning inorganic sulfur to the environment.



Sulfur cycle

This figure summarizes the sulfur cycle. Note that specific groups of prokaryotes each may participate in every step in the cycle. (credit: modification of work by NOAA)

Note:

- Which groups of microbes carry out the sulfur cycle?

Other Biogeochemical Cycles

Beyond their involvement in the carbon, nitrogen, and sulfur cycles, prokaryotes are involved in other biogeochemical cycles as well. Like the carbon, nitrogen, and sulfur cycles, several of these additional biogeochemical cycles, such as the iron (Fe), manganese (Mn), and chromium (Cr) cycles, also involve redox chemistry, with prokaryotes playing roles in both oxidation and reduction. Several other elements undergo chemical cycles that do not involve redox chemistry. Examples of these are phosphorus (P), calcium (Ca), and silica (Si) cycles. The cycling of these elements is particularly important in oceans because large quantities of these elements are incorporated into the exoskeletons of marine organisms. These biogeochemical cycles do not involve redox chemistry but instead involve fluctuations in the solubility of compounds containing calcium, phosphorous, and silica. The overgrowth of naturally occurring microbial communities is typically limited by the availability of nitrogen (as previously mentioned), phosphorus, and iron. Human activities introducing excessive amounts of iron, nitrogen, or phosphorus (typically from detergents) may lead to eutrophication.

Bioremediation

Microbial **bioremediation** leverages microbial metabolism to remove **xenobiotics** or other pollutants. Xenobiotics are compounds synthesized by humans and introduced into the environment in much higher concentrations than would naturally occur. Such environmental contamination may involve adhesives, dyes, flame retardants, lubricants, oil and petroleum products, organic solvents, pesticides, and products of the combustion of gasoline and oil. Many xenobiotics resist breakdown, and some accumulate in the food chain after being consumed or absorbed by fish and wildlife, which, in turn, may be eaten by humans. Of particular concern are contaminants like polycyclic aromatic hydrocarbon (PAH), a carcinogenic xenobiotic found in crude oil, and trichloroethylene (TCE), a common groundwater contaminant.

Bioremediation processes can be categorized as *in situ* or *ex situ*. Bioremediation conducted at the site of contamination is called *in situ* bioremediation and does not involve movement of contaminated material. In contrast, *ex situ* bioremediation involves the removal of contaminated

material from the original site so that it can be treated elsewhere, typically in a large, lined pit where conditions are optimized for degradation of the contaminant.

Some bioremediation processes rely on microorganisms that are indigenous to the contaminated site or material. Enhanced bioremediation techniques, which may be applied to either in situ or ex situ processing, involve the addition of nutrients and/or air to encourage the growth of pollution-degrading microbes; they may also involve the addition of non-native microbes known for their ability to degrade contaminants. For example, certain bacteria of the genera *Rhodococcus* and *Pseudomonas* are known for their ability to degrade many environmental contaminants, including aromatic compounds like those found in oil, down to CO₂. The genes encoding their degradatory enzymes are commonly found on plasmids. Others, like *Alcanivorax borkumensis*, produce surfactants that are useful in the solubilization of the hydrophobic molecules found in oil, making them more accessible to other microbes for degradation.

Note:

- Compare and contrast the benefits of in situ and ex situ bioremediation.

Note:

Resolution

Although there is a DNA test specific for *Neisseria meningitidis*, it is not practical for use in some developing countries because it requires expensive equipment and a high level of expertise to perform. The hospital in Banjul was not equipped to perform DNA testing. Biochemical testing, however, is much less expensive and is still effective for microbial identification.

Fortunately for Hannah, her symptoms began to resolve with antibiotic therapy. Patients who survive bacterial meningitis often suffer from long-

term complications such as brain damage, hearing loss, and seizures, but after several weeks of recovery, Hannah did not seem to be exhibiting any long-term effects and her behavior returned to normal. Because of her age, her parents were advised to monitor her closely for any signs of developmental issues and have her regularly evaluated by her pediatrician. *N. meningitidis* is found in the normal respiratory microbiota in 10%–20% of the human population. [footnote] In most cases, it does not cause disease, but for reasons not fully understood, the bacterium can sometimes invade the bloodstream and cause infections in other areas of the body, including the brain. The disease is more common in infants and children, like Hannah.

Centers for Disease Control and Prevention. “Meningococcal Disease: Causes and Transmission.”

<http://www.cdc.gov/meningococcal/about/causes-transmission.html>. Accessed September 12, 2016.

The prevalence of meningitis caused by *N. meningitidis* is particularly high in the so-called meningitis belt, a region of sub-Saharan African that includes 26 countries stretching from Senegal to Ethiopia ([link]). The reasons for this high prevalence are not clear, but several factors may contribute to higher rates of transmission, such as the dry, dusty climate; overcrowding and low standards of living; and the relatively low immunocompetence and nutritional status of the population. [footnote] A vaccine against four bacterial strains of *N. meningitidis* is available.

Vaccination is recommended for 11- and 12-year-old children, with a booster at age 16 years. Vaccination is also recommended for young people who live in close quarters with others (e.g., college dormitories, military barracks), where the disease is more easily transmitted. Travelers visiting the “meningitis belt” should also be vaccinated, especially during the dry season (December through June) when the prevalence is highest. [footnote] [footnote]

Centers for Disease Control and Prevention. “Meningococcal Disease in Other Countries.” <http://www.cdc.gov/meningococcal/global.html>.

Accessed September 12, 2016.

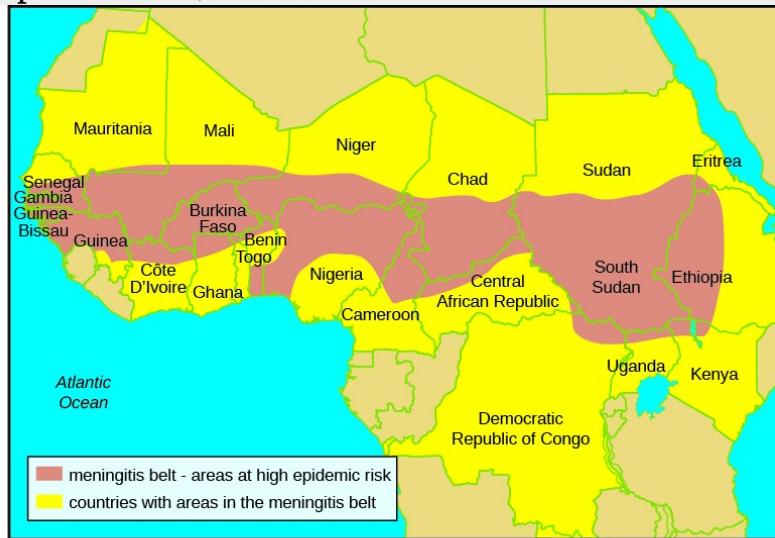
Centers for Disease Control and Prevention. “Health Information for Travelers to the Gambia: Traveler View.”

<http://wwwnc.cdc.gov/travel/destinations/traveler/none/the-gambia>. Accessed September 12, 2016.

Centers for Disease Control and Prevention. “Meningococcal: Who Needs to Be Vaccinated?” <http://www.cdc.gov/vaccines/vpd-vac/mening/who-vaccinate.htm>. Accessed September 12, 2016.



(a)



(b)

(a) *Neisseria meningitidis* is a gram-negative diplococcus, as shown in this gram-stained sample. (b) The “meningitis belt” is the area of sub-Saharan Africa with high prevalence of meningitis caused by *N. meningitidis*. (credit a, b: modification of work by Centers for Disease Control and Prevention)

Go back to the [previous](#) Clinical Focus box.

Key Concepts and Summary

- The recycling of inorganic matter between living organisms and their nonliving environment is called a **biogeochemical cycle**. Microbes play significant roles in these cycles.
- In the **carbon cycle**, heterotrophs degrade reduced organic molecule to produce carbon dioxide, whereas autotrophs fix carbon dioxide to produce organics. **Methanogens** typically form methane by using CO₂

as a final electron acceptor during anaerobic respiration; methanotrophs oxidize the methane, using it as their carbon source.

- In the **nitrogen cycle**, nitrogen-fixing bacteria convert atmospheric nitrogen into ammonia (ammonification). The ammonia can then be oxidized to nitrite and nitrate (nitrification). Nitrates can then be assimilated by plants. Soil bacteria convert nitrate back to nitrogen gas (denitrification).
- In **sulfur cycling**, many anoxygenic photosynthesizers and chemoautotrophs use hydrogen sulfide as an electron donor, producing elemental sulfur and then sulfate; sulfate-reducing bacteria and archaea then use sulfate as a final electron acceptor in anaerobic respiration, converting it back to hydrogen sulfide.
- Human activities that introduce excessive amounts of naturally limited nutrients (like iron, nitrogen, or phosphorus) to aquatic systems may lead to eutrophication.
- Microbial **bioremediation** is the use of microbial metabolism to remove or degrade **xenobiotics** and other environmental contaminants and pollutants. Enhanced bioremediation techniques may involve the introduction of non-native microbes specifically chosen or engineered for their ability to degrade contaminants.

Multiple Choice

Exercise:

Problem:

Which of the following is the group of archaea that can use CO_2 as their final electron acceptor during anaerobic respiration, producing CH_4 ?

- A. methylotrophs
- B. methanotrophs
- C. methanogens
- D. anoxygenic photosynthesizers

Solution:

C

Exercise:

Problem:

Which of the following processes is not involved in the conversion of organic nitrogen to nitrogen gas?

- A. nitrogen fixation
 - B. ammonification
 - C. nitrification
 - D. denitrification
-

Solution:

A

Exercise:

Problem:

Which of the following processes produces hydrogen sulfide?

- A. anoxygenic photosynthesis
 - B. oxygenic photosynthesis
 - C. anaerobic respiration
 - D. chemoaautrophy
-

Solution:

C

Exercise:

Problem:

The biogeochemical cycle of which of the following elements is based on changes in solubility rather than redox chemistry?

- A. carbon
 - B. sulfur
 - C. nitrogen
 - D. phosphorus
-

Solution:

D

Fill in the Blank

Exercise:

Problem:

The molecule central to the carbon cycle that is exchanged within and between ecosystems, being produced by heterotrophs and used by autotrophs, is _____.

Solution:

carbon dioxide

Exercise:

Problem:

The use of microbes to remove pollutants from a contaminated system is called _____.

Solution:

bioremediation

True/False

Exercise:

Problem:

There are many naturally occurring microbes that have the ability to degrade several of the compounds found in oil.

Solution:

True

Short Answer**Exercise:****Problem:**

Why must autotrophic organisms also respire or ferment in addition to fixing CO₂?

Exercise:

Problem: How can human activity lead to eutrophication?

Critical Thinking**Exercise:****Problem:**

In considering the symbiotic relationship between *Rhizobium* species and their plant hosts, what metabolic activity does each organism perform that benefits the other member of the pair?

Microbial Growth - Introduction

class="introduction"

Medical devices that are inserted into a patient's body often become contaminated

with a thin biofilm of microorganism s enmeshed in the sticky material they secrete. The

electron micrograph (left) shows the inside walls of an in-dwelling catheter.

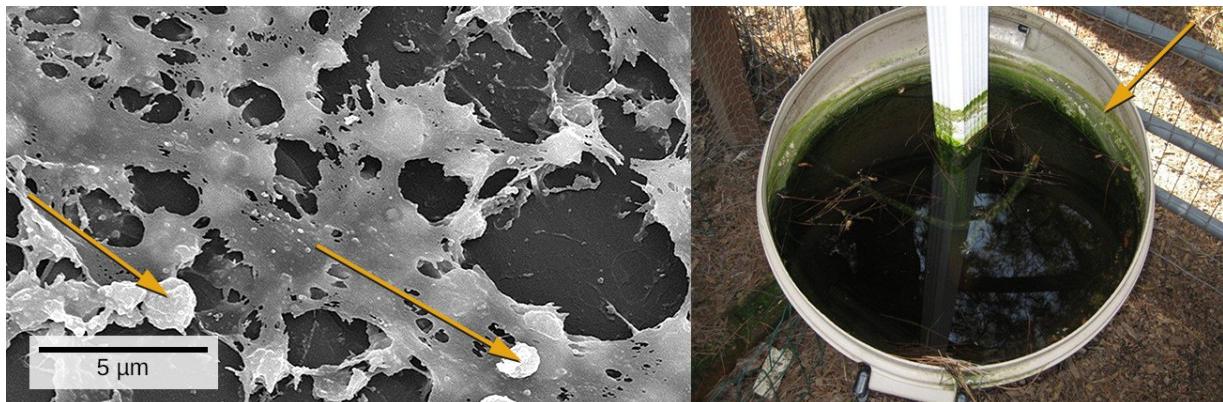
Arrows point to the round cells

of *Staphylococcus aureus* bacteria attached to the

layers of extracellular substrate. The garbage can (right) served

as a rain collector. The arrow points to a green biofilm

on the sides of
the container.
(credit left:
modification of
work by
Centers for
Disease
Control and
Prevention;
credit right:
modification of
work by
NASA)



We are all familiar with the slimy layer on a pond surface or that makes rocks slippery. These are examples of biofilms—microorganisms embedded in thin layers of matrix material ([\[link\]](#)). Biofilms were long considered random assemblages of cells and had little attention from researchers. Recently, progress in visualization and biochemical methods has revealed that biofilms are an organized ecosystem within which many cells, usually of different species of bacteria, fungi, and algae, interact through cell signaling and coordinated responses. The biofilm provides a protected environment in harsh conditions and aids colonization by microorganisms. Biofilms also have clinical importance. They form on medical devices, resist routine cleaning and sterilization, and cause health-acquired infections. Within the body, biofilms form on the teeth as plaque, in the

lungs of patients with cystic fibrosis, and on the cardiac tissue of patients with endocarditis. The slime layer helps protect the cells from host immune defenses and antibiotic treatments.

Studying biofilms requires new approaches. Because of the cells' adhesion properties, many of the methods for culturing and counting cells that are explored in this chapter are not easily applied to biofilms. This is the beginning of a new era of challenges and rewarding insight into the ways that microorganisms grow and thrive in nature.

How Microbes Grow

LEARNING OBJECTIVES

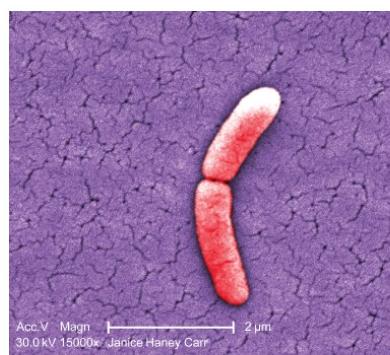
- Define the generation time for growth based on binary fission
- Identify and describe the activities of microorganisms undergoing typical phases of binary fission (simple cell division) in a growth curve
- Explain several laboratory methods used to determine viable and total cell counts in populations undergoing exponential growth
- Describe examples of cell division not involving binary fission, such as budding or fragmentation
- Describe the formation and characteristics of biofilms
- Identify health risks associated with biofilms and how they are addressed
- Describe quorum sensing and its role in cell-to-cell communication and coordination of cellular activities

The bacterial cell cycle involves the formation of new cells through the replication of DNA and partitioning of cellular components into two daughter cells. In prokaryotes, reproduction is always asexual, although extensive genetic recombination in the form of horizontal gene transfer takes place, as will be explored in a different chapter. Most bacteria have a single circular chromosome; however, some exceptions exist. For example, *Borrelia burgdorferi*, the causative agent of Lyme disease, has a linear chromosome.

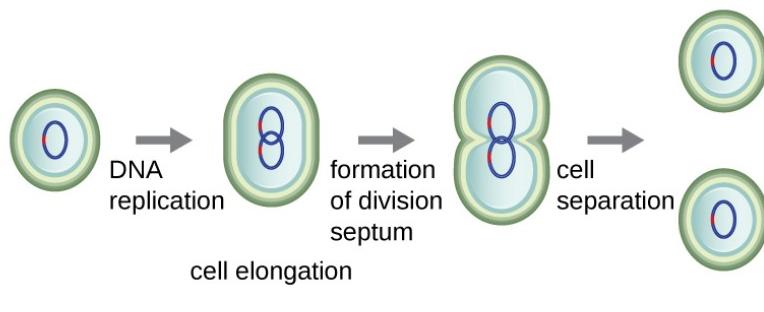
Binary Fission

The most common mechanism of cell replication in bacteria is a process called **binary fission**, which is depicted in [\[link\]](#). Before dividing, the cell grows and increases its number of cellular components. Next, the replication of DNA starts at a location on the circular chromosome called the origin of replication, where the chromosome is attached to the inner cell membrane. Replication continues in opposite directions along the chromosome until the terminus is reached.

The center of the enlarged cell constricts until two daughter cells are formed, each offspring receiving a complete copy of the parental genome and a division of the cytoplasm (cytokinesis). This process of cytokinesis and cell division is directed by a protein called FtsZ. FtsZ assembles into a Z ring on the cytoplasmic membrane ([\[link\]](#)). The Z ring is anchored by FtsZ-binding proteins and defines the division plane between the two daughter cells. Additional proteins required for cell division are added to the Z ring to form a structure called the divisome. The divisome activates to produce a peptidoglycan cell wall and build a **septum** that divides the two daughter cells. The daughter cells are separated by the division septum, where all of the cells' outer layers (the cell wall and outer membranes, if present) must be remodeled to complete division. For example, we know that specific enzymes break bonds between the monomers in peptidoglycans and allow addition of new subunits along the division septum.



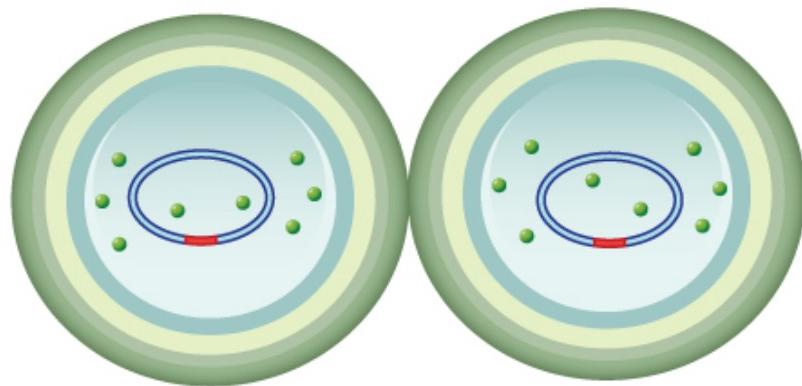
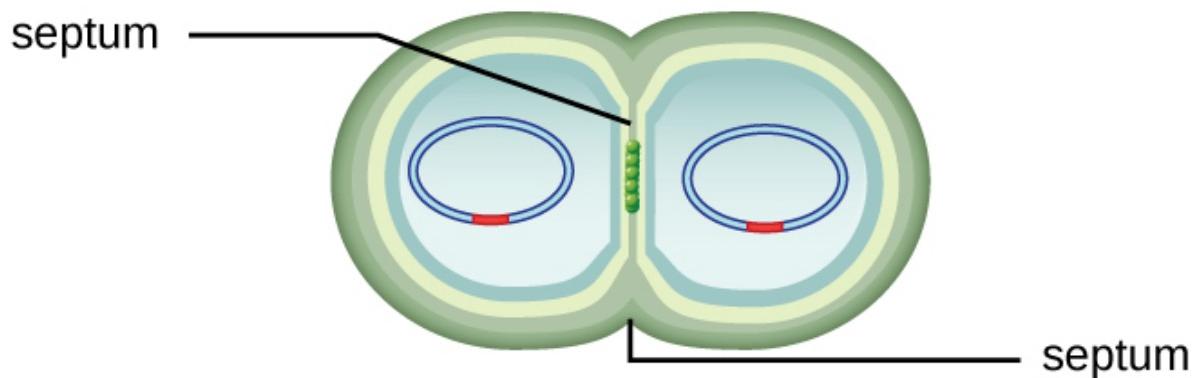
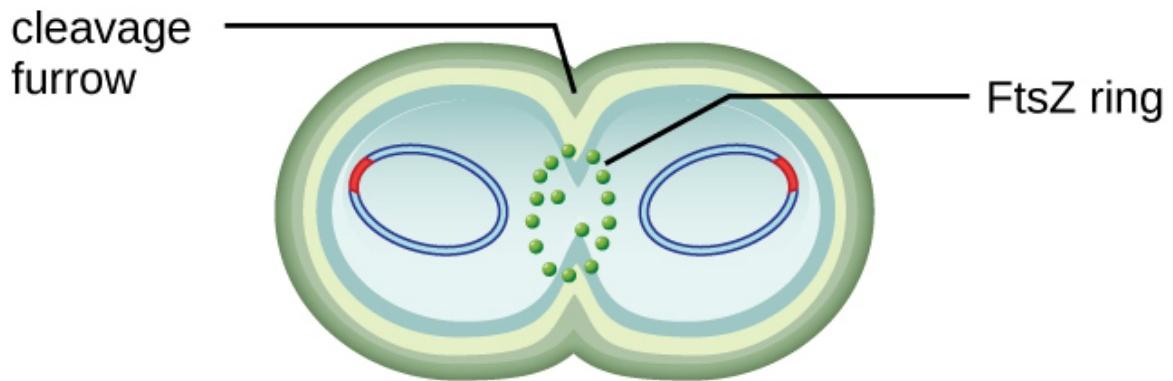
(a)



(b)

(a) The electron micrograph depicts two cells of *Salmonella typhimurium* after a binary fission event. (b) Binary fission in bacteria

starts with the replication of DNA as the cell elongates. A division septum forms in the center of the cell. Two daughter cells of similar size form and separate, each receiving a copy of the original chromosome. (credit a: modification of work by Centers for Disease Control and Prevention)



FtsZ proteins assemble to form a Z ring that is anchored to the plasma membrane. The Z ring pinches the cell envelope to separate the cytoplasm of the new cells.

Note:

- What is the name of the protein that assembles into a Z ring to initiate cytokinesis and cell division?

Generation Time

In eukaryotic organisms, the generation time is the time between the same points of the life cycle in two successive generations. For example, the typical generation time for the human population is 25 years. This definition is not practical for bacteria, which may reproduce rapidly or remain dormant for thousands of years. In prokaryotes (Bacteria and Archaea), the **generation time** is also called the **doubling time** and is defined as the time it takes for the population to double through one round of binary fission. Bacterial doubling times vary enormously. Whereas *Escherichia coli* can double in as little as 20 minutes under optimal growth conditions in the laboratory, bacteria of the same species may need several days to double in especially harsh environments. Most pathogens grow rapidly, like *E. coli*, but there are exceptions. For example, *Mycobacterium tuberculosis*, the causative agent of tuberculosis, has a generation time of between 15 and 20 hours. On the other hand, *M. leprae*, which causes Hansen's disease (leprosy), grows much more slowly, with a doubling time of 14 days.

Note:

Calculating Number of Cells

It is possible to predict the number of cells in a population when they divide by binary fission at a constant rate. As an example, consider what

happens if a single cell divides every 30 minutes for 24 hours. The diagram in [\[link\]](#) shows the increase in cell numbers for the first three generations. The number of cells increases exponentially and can be expressed as 2^n , where n is the number of generations. If cells divide every 30 minutes, after 24 hours, 48 divisions would have taken place. If we apply the formula 2^n , where n is equal to 48, the single cell would give rise to 2^{48} or 281,474,976,710,656 cells at 48 generations (24 hours). When dealing with such huge numbers, it is more practical to use scientific notation.

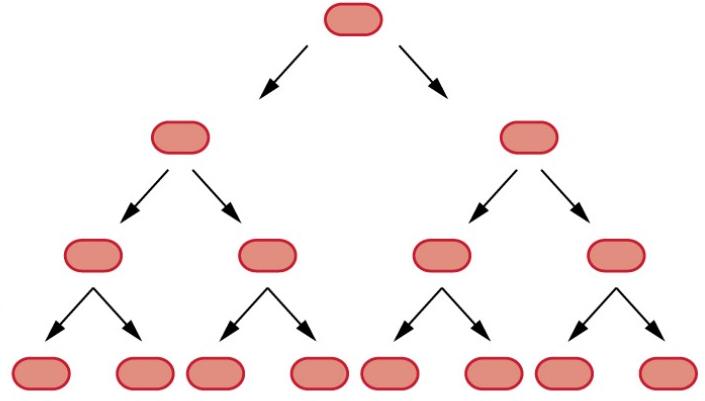
Therefore, we express the number of cells as 2.8×10^{14} cells.

In our example, we used one cell as the initial number of cells. For any number of starting cells, the formula is adapted as follows:

Equation:

$$N_n = N_0 2^n$$

N_n is the number of cells at any generation n , N_0 is the initial number of cells, and n is the number of generations.

Number of generations (n)	Number of cells	Each division adds two new cells
0	1	
1	2	
2	4	
3	8	

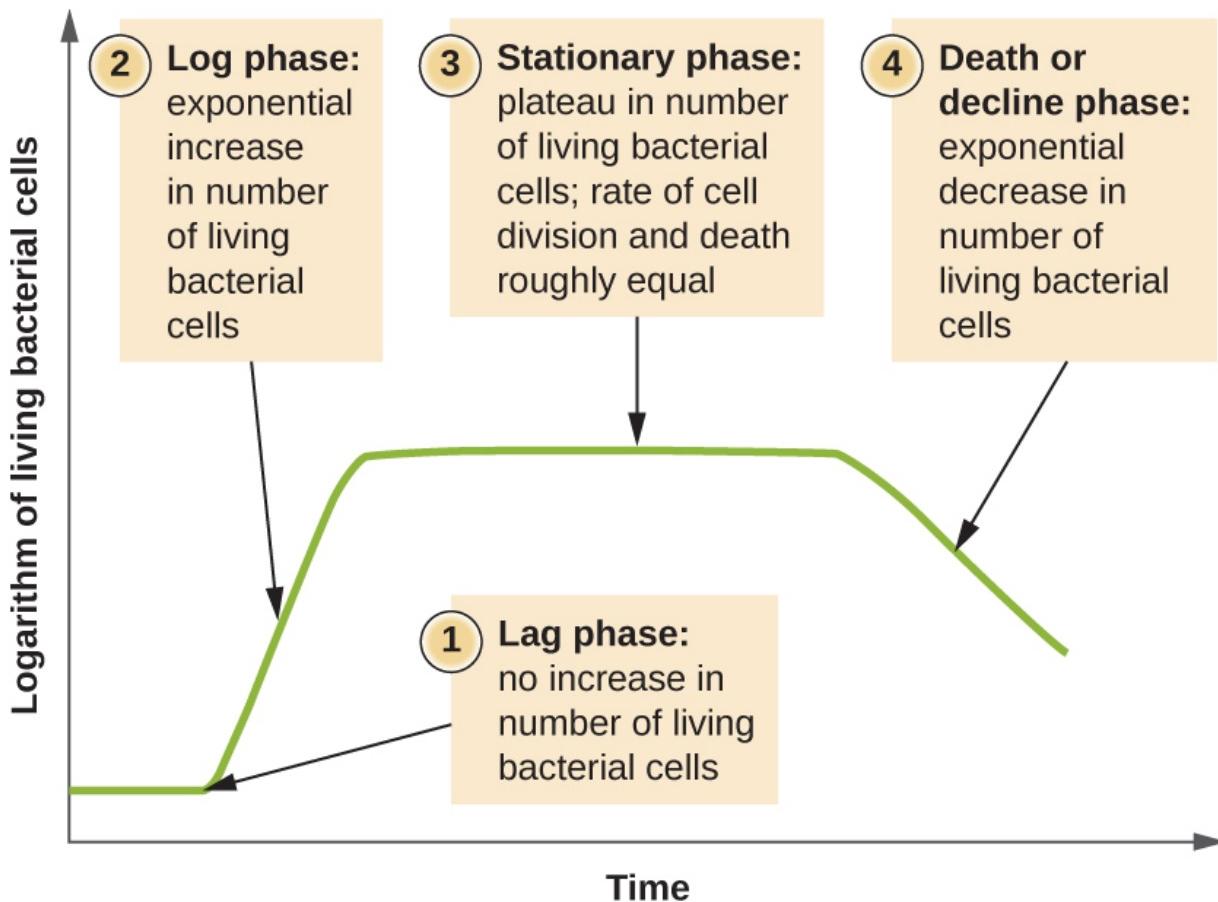
The parental cell divides and gives rise to two daughter cells. Each of the daughter cells, in turn, divides, giving a total of four cells in the second generation and eight cells in the third generation. Each division doubles the number of cells.

Note:

- With a doubling time of 30 minutes and a starting population size of 1×10^5 cells, how many cells will be present after 2 hours, assuming no cell death?

The Growth Curve

Microorganisms grown in closed culture (also known as a batch culture), in which no nutrients are added and most waste is not removed, follow a reproducible growth pattern referred to as the **growth curve**. An example of a batch culture in nature is a pond in which a small number of cells grow in a closed environment. The **culture density** is defined as the number of cells per unit volume. In a closed environment, the culture density is also a measure of the number of cells in the population. Infections of the body do not always follow the growth curve, but correlations can exist depending upon the site and type of infection. When the number of live cells is plotted against time, distinct phases can be observed in the curve ([\[link\]](#)).



The growth curve of a bacterial culture is represented by the logarithm of the number of live cells plotted as a function of time. The graph can be divided into four phases according to the slope, each of which matches events in the cell. The four phases are lag, log, stationary, and death.

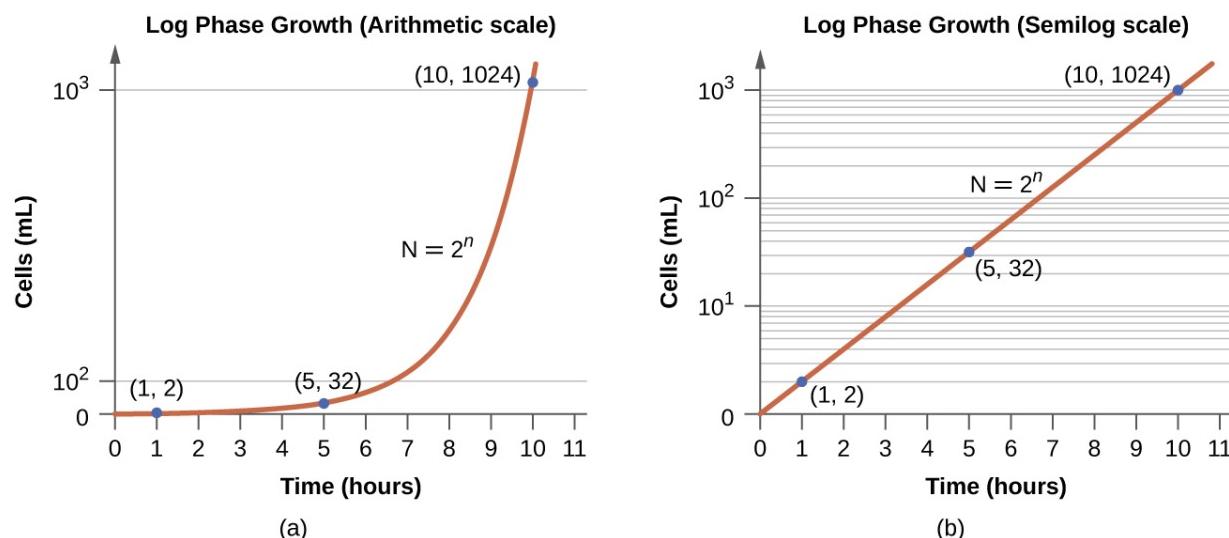
The Lag Phase

The beginning of the growth curve represents a small number of cells, referred to as an **inoculum**, that are added to a fresh **culture medium**, a nutritional broth that supports growth. The initial phase of the growth curve is called the **lag phase**, during which cells are gearing up for the next phase of growth. The number of cells does not change during the lag phase;

however, cells grow larger and are metabolically active, synthesizing proteins needed to grow within the medium. If any cells were damaged or shocked during the transfer to the new medium, repair takes place during the lag phase. The duration of the lag phase is determined by many factors, including the species and genetic make-up of the cells, the composition of the medium, and the size of the original inoculum.

The Log Phase

In the **logarithmic (log) growth phase**, sometimes called exponential growth phase, the cells are actively dividing by binary fission and their number increases exponentially. For any given bacterial species, the generation time under specific growth conditions (nutrients, temperature, pH, and so forth) is genetically determined, and this generation time is called the **intrinsic growth rate**. During the log phase, the relationship between time and number of cells is not linear but exponential; however, the growth curve is often plotted on a semilogarithmic graph, as shown in [\[link\]](#), which gives the appearance of a linear relationship.



Both graphs illustrate population growth during the log phase for a bacterial sample with an initial population of one cell and a doubling

time of 1 hour. (a) When plotted on an arithmetic scale, the growth rate resembles a curve. (b) When plotted on a semilogarithmic scale (meaning the values on the y -axis are logarithmic), the growth rate appears linear.

Cells in the log phase show constant growth rate and uniform metabolic activity. For this reason, cells in the log phase are preferentially used for industrial applications and research work. The log phase is also the stage where bacteria are the most susceptible to the action of disinfectants and common antibiotics that affect protein, DNA, and cell-wall synthesis.

Stationary Phase

As the number of cells increases through the log phase, several factors contribute to a slowing of the growth rate. Waste products accumulate and nutrients are gradually used up. In addition, gradual depletion of oxygen begins to limit aerobic cell growth. This combination of unfavorable conditions slows and finally stalls population growth. The total number of live cells reaches a plateau referred to as the **stationary phase** ([\[link\]](#)). In this phase, the number of new cells created by cell division is now equivalent to the number of cells dying; thus, the total population of living cells is relatively stagnant. The culture density in a stationary culture is constant. The culture's carrying capacity, or maximum culture density, depends on the types of microorganisms in the culture and the specific conditions of the culture; however, carrying capacity is constant for a given organism grown under the same conditions.

During the stationary phase, cells switch to a survival mode of metabolism. As growth slows, so too does the synthesis of peptidoglycans, proteins, and nucleic-acids; thus, stationary cultures are less susceptible to antibiotics that disrupt these processes. In bacteria capable of producing endospores, many cells undergo sporulation during the stationary phase. Secondary metabolites, including antibiotics, are synthesized in the stationary phase. In certain pathogenic bacteria, the stationary phase is also associated with the

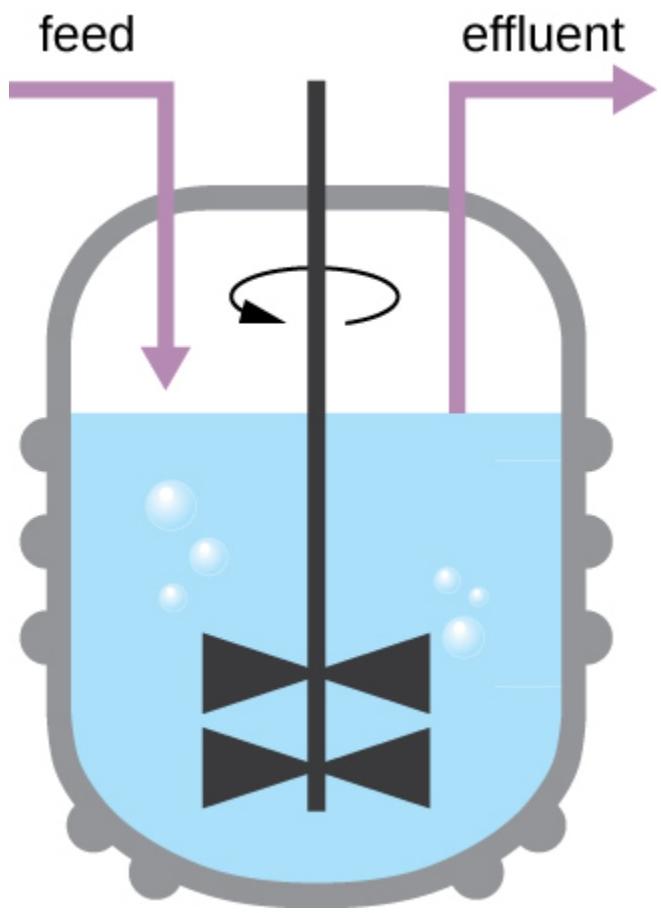
expression of virulence factors, products that contribute to a microbe's ability to survive, reproduce, and cause disease in a host organism. For example, quorum sensing in *Staphylococcus aureus* initiates the production of enzymes that can break down human tissue and cellular debris, clearing the way for bacteria to spread to new tissue where nutrients are more plentiful.

The Death Phase

As a culture medium accumulates toxic waste and nutrients are exhausted, cells die in greater and greater numbers. Soon, the number of dying cells exceeds the number of dividing cells, leading to an exponential decrease in the number of cells ([\[link\]](#)). This is the aptly named **death phase**, sometimes called the decline phase. Many cells lyse and release nutrients into the medium, allowing surviving cells to maintain viability and form endospores. A few cells, the so-called **persisters**, are characterized by a slow metabolic rate. Persister cells are medically important because they are associated with certain chronic infections, such as tuberculosis, that do not respond to antibiotic treatment.

Sustaining Microbial Growth

The growth pattern shown in [\[link\]](#) takes place in a closed environment; nutrients are not added and waste and dead cells are not removed. In many cases, though, it is advantageous to maintain cells in the logarithmic phase of growth. One example is in industries that harvest microbial products. A chemostat ([\[link\]](#)) is used to maintain a continuous culture in which nutrients are supplied at a steady rate. A controlled amount of air is mixed in for aerobic processes. Bacterial suspension is removed at the same rate as nutrients flow in to maintain an optimal growth environment.



A chemostat is a culture vessel fitted with an opening to add nutrients (feed) and an outlet to remove contents (effluent), effectively diluting toxic wastes and dead cells. The addition and removal of fluids is adjusted to maintain the culture in the logarithmic phase of growth. If aerobic bacteria are grown, suitable oxygen levels are maintained.

Note:

- During which phase does growth occur at the fastest rate?
- Name two factors that limit microbial growth.

Measurement of Bacterial Growth

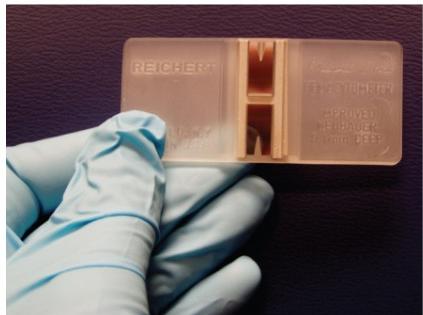
Estimating the number of bacterial cells in a sample, known as a bacterial count, is a common task performed by microbiologists. The number of bacteria in a clinical sample serves as an indication of the extent of an infection. Quality control of drinking water, food, medication, and even cosmetics relies on estimates of bacterial counts to detect contamination and prevent the spread of disease. Two major approaches are used to measure cell number. The direct methods involve counting cells, whereas the indirect methods depend on the measurement of cell presence or activity without actually counting individual cells. Both direct and indirect methods have advantages and disadvantages for specific applications.

Direct Cell Count

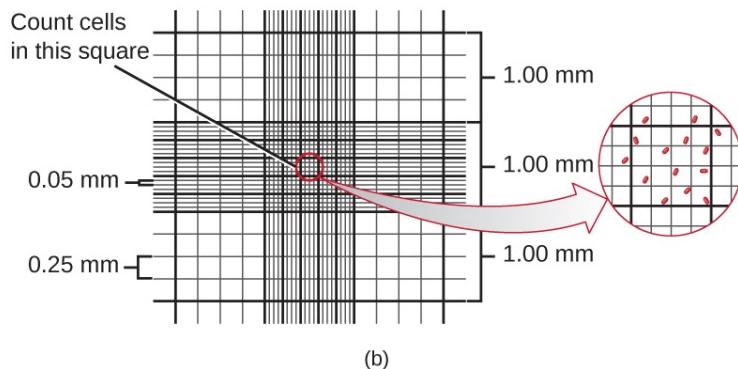
Direct cell count refers to counting the cells in a liquid culture or colonies on a plate. It is a direct way of estimating how many organisms are present in a sample. Let's look first at a simple and fast method that requires only a specialized slide and a compound microscope.

The simplest way to count bacteria is called the **direct microscopic cell count**, which involves transferring a known volume of a culture to a calibrated slide and counting the cells under a light microscope. The calibrated slide is called a **Petroff-Hausser chamber** ([\[link\]](#)) and is similar to a hemocytometer used to count red blood cells. The central area of the counting chamber is etched into squares of various sizes. A sample of the culture suspension is added to the chamber under a coverslip that is placed at a specific height from the surface of the grid. It is possible to estimate the

concentration of cells in the original sample by counting individual cells in a number of squares and determining the volume of the sample observed. The area of the squares and the height at which the coverslip is positioned are specified for the chamber. The concentration must be corrected for dilution if the sample was diluted before enumeration.



(a)

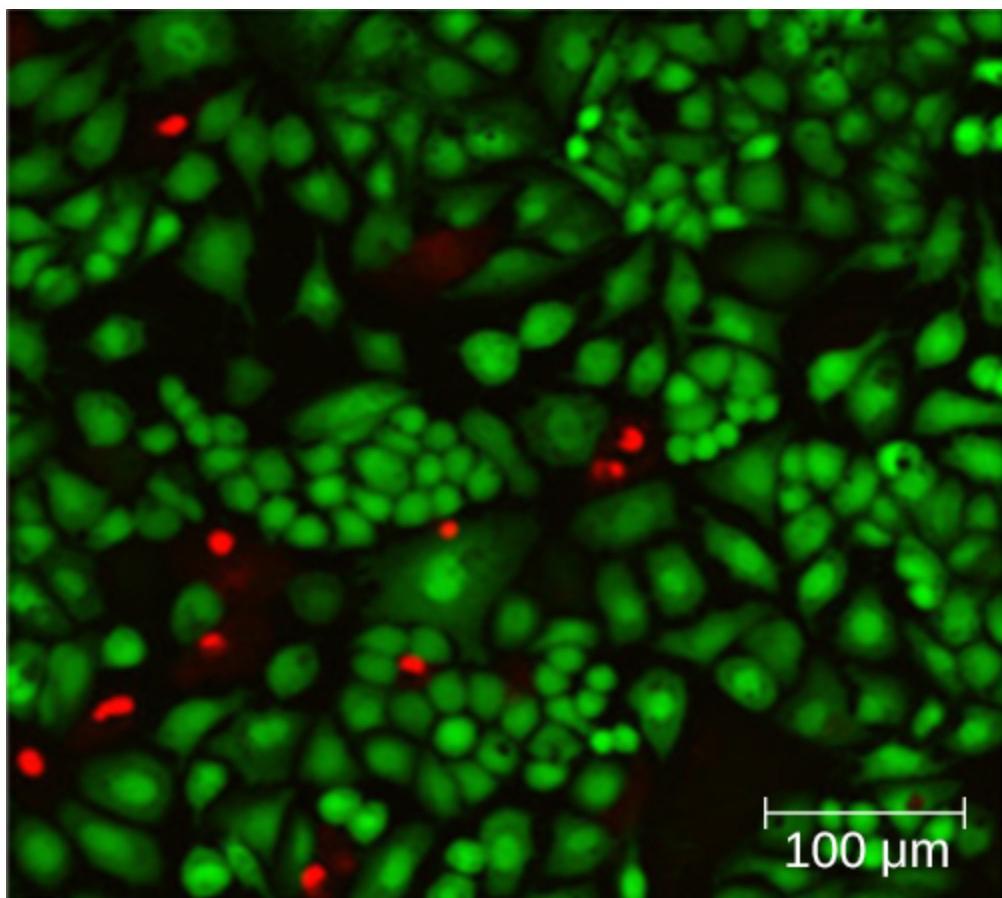


(b)

(a) A Petroff-Hausser chamber is a special slide designed for counting the bacterial cells in a measured volume of a sample. A grid is etched on the slide to facilitate precision in counting. (b) This diagram illustrates the grid of a Petroff-Hausser chamber, which is made up of squares of known areas. The enlarged view shows the square within which bacteria (red cells) are counted. If the coverslip is 0.2 mm above the grid and the square has an area of 0.04 mm^2 , then the volume is 0.008 mm^3 , or 0.000008 mL . Since there are 10 cells inside the square, the density of bacteria is $10 \text{ cells}/0.000008 \text{ mL}$, which equates to $1,250,000 \text{ cells/mL}$. (credit a: modification of work by Jeffrey M. Vinocur)

Cells in several small squares must be counted and the average taken to obtain a reliable measurement. The advantages of the chamber are that the method is easy to use, relatively fast, and inexpensive. On the downside, the counting chamber does not work well with dilute cultures because there may not be enough cells to count.

Using a counting chamber does not necessarily yield an accurate count of the number of live cells because it is not always possible to distinguish between live cells, dead cells, and debris of the same size under the microscope. However, newly developed fluorescence staining techniques make it possible to distinguish viable and dead bacteria. These viability stains (or live stains) bind to nucleic acids, but the primary and secondary stains differ in their ability to cross the cytoplasmic membrane. The primary stain, which fluoresces green, can penetrate intact cytoplasmic membranes, staining both live and dead cells. The secondary stain, which fluoresces red, can stain a cell only if the cytoplasmic membrane is considerably damaged. Thus, live cells fluoresce green because they only absorb the green stain, whereas dead cells appear red because the red stain displaces the green stain on their nucleic acids ([\[link\]](#)).

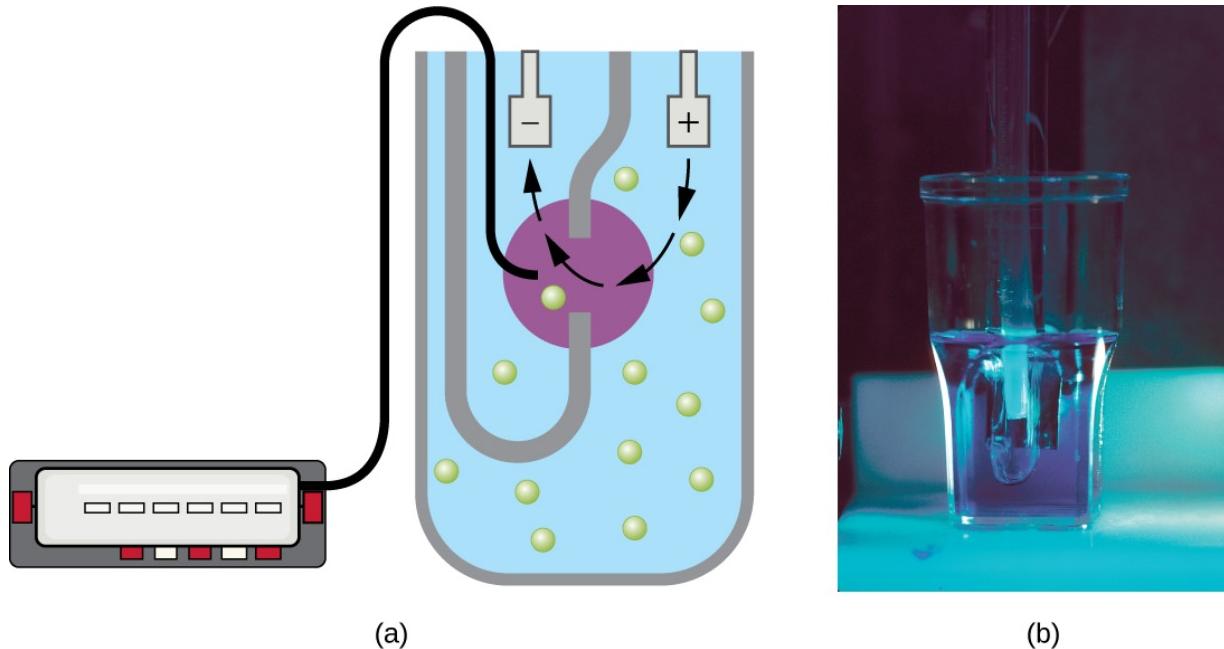


Fluorescence staining can be used to differentiate

between viable and dead bacterial cells in a sample for purposes of counting. Viable cells are stained green, whereas dead cells are stained red. (credit: modification of work by Panseri S, Cunha C, D'Alessandro T, Sandri M, Giavaresi G, Maracci M, Hung CT, Tampieri A)

Another technique uses an electronic cell counting device (Coulter counter) to detect and count the changes in electrical resistance in a saline solution. A glass tube with a small opening is immersed in an electrolyte solution. A first electrode is suspended in the glass tube. A second electrode is located outside of the tube. As cells are drawn through the small aperture in the glass tube, they briefly change the resistance measured between the two electrodes and the change is recorded by an electronic sensor ([\[link\]](#)); each resistance change represents a cell. The method is rapid and accurate within a range of concentrations; however, if the culture is too concentrated, more than one cell may pass through the aperture at any given time and skew the results. This method also does not differentiate between live and dead cells.

Direct counts provide an estimate of the total number of cells in a sample. However, in many situations, it is important to know the number of live, or **viable**, cells. Counts of live cells are needed when assessing the extent of an infection, the effectiveness of antimicrobial compounds and medication, or contamination of food and water.



A Coulter counter is an electronic device that counts cells. It measures the change in resistance in an electrolyte solution that takes place when a cell passes through a small opening in the inside container wall. A detector automatically counts the number of cells passing through the opening. (credit b: modification of work by National Institutes of Health)

Note:

- Why would you count the number of cells in more than one square in the Petroff-Hausser chamber to estimate cell numbers?
- In the viability staining method, why do dead cells appear red?

Plate Count

The **viable plate count**, or simply plate count, is a count of viable or live cells. It is based on the principle that viable cells replicate and give rise to visible colonies when incubated under suitable conditions for the specimen. The results are usually expressed as **colony-forming units** per milliliter (CFU/mL) rather than cells per milliliter because more than one cell may have landed on the same spot to give rise to a single colony. Furthermore, samples of bacteria that grow in clusters or chains are difficult to disperse and a single colony may represent several cells. Some cells are described as viable but nonculturable and will not form colonies on solid media. For all these reasons, the viable plate count is considered a low estimate of the actual number of live cells. These limitations do not detract from the usefulness of the method, which provides estimates of live bacterial numbers.

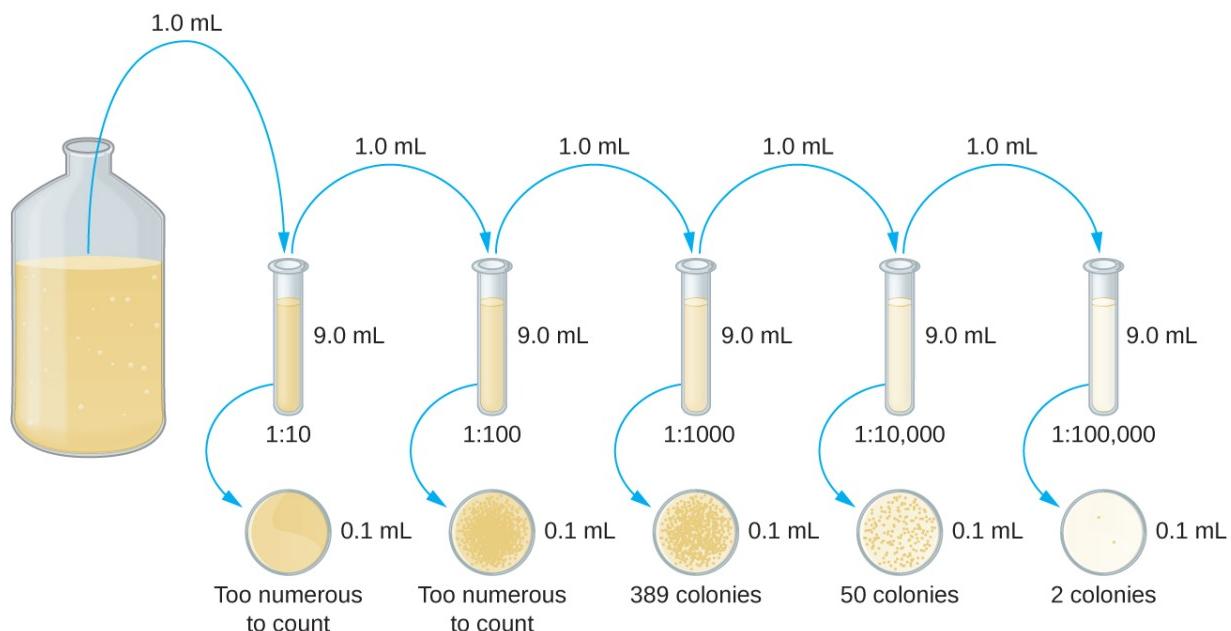
Microbiologists typically count plates with 30–300 colonies. Samples with too few colonies (<30) do not give statistically reliable numbers, and overcrowded plates (>300 colonies) make it difficult to accurately count individual colonies. Also, counts in this range minimize occurrences of more than one bacterial cell forming a single colony. Thus, the calculated CFU is closer to the true number of live bacteria in the population.

There are two common approaches to inoculating plates for viable counts: the pour plate and the spread plate methods. Although the final inoculation procedure differs between these two methods, they both start with a serial dilution of the culture.

Serial Dilution

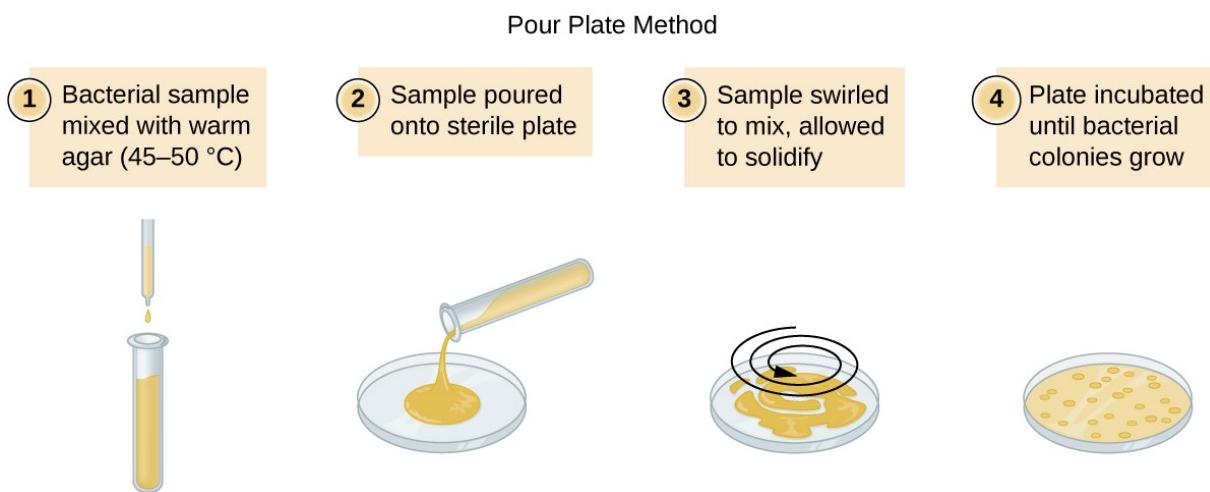
The **serial dilution** of a culture is an important first step before proceeding to either the pour plate or spread plate method. The goal of the serial dilution process is to obtain plates with CFUs in the range of 30–300, and the process usually involves several dilutions in multiples of 10 to simplify calculation. The number of serial dilutions is chosen according to a preliminary estimate of the culture density. [\[link\]](#) illustrates the serial dilution method.

A fixed volume of the original culture, 1.0 mL, is added to and thoroughly mixed with the first dilution tube solution, which contains 9.0 mL of sterile broth. This step represents a dilution factor of 10, or 1:10, compared with the original culture. From this first dilution, the same volume, 1.0 mL, is withdrawn and mixed with a fresh tube of 9.0 mL of dilution solution. The dilution factor is now 1:100 compared with the original culture. This process continues until a series of dilutions is produced that will bracket the desired cell concentration for accurate counting. From each tube, a sample is plated on solid medium using either the **pour plate method** ([\[link\]](#)) or the **spread plate method** ([\[link\]](#)). The plates are incubated until colonies appear. Two to three plates are usually prepared from each dilution and the numbers of colonies counted on each plate are averaged. In all cases, thorough mixing of samples with the dilution medium (to ensure the cell distribution in the tube is random) is paramount to obtaining reliable results.



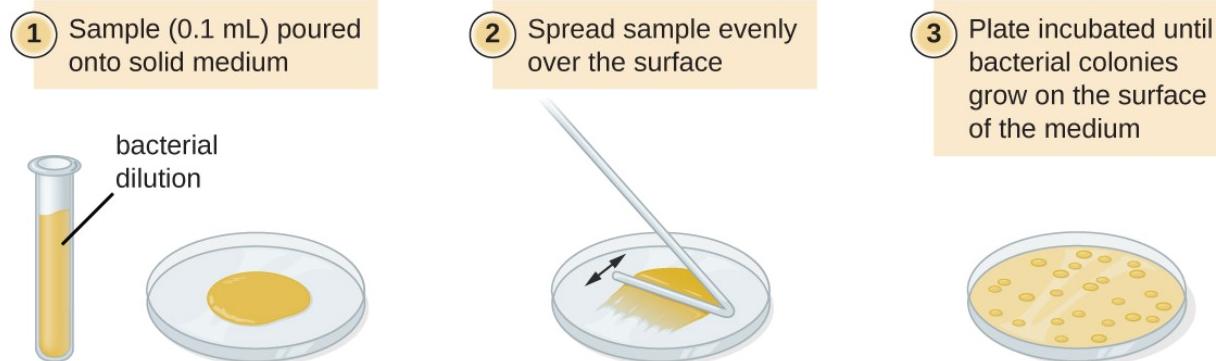
Serial dilution involves diluting a fixed volume of cells mixed with dilution solution using the previous dilution as an inoculum. The result is dilution of the original culture by an exponentially growing factor. (credit: modification of work by “Leberechtc”/Wikimedia Commons)

The dilution factor is used to calculate the number of cells in the original cell culture. In our example, an average of 50 colonies was counted on the plates obtained from the 1:10,000 dilution. Because only 0.1 mL of suspension was pipetted on the plate, the multiplier required to reconstitute the original concentration is $10 \times 10,000$. The number of CFU per mL is equal to $50 \times 100 \times 10,000 = 5,000,000$. The number of bacteria in the culture is estimated as 5 million cells/mL. The colony count obtained from the 1:1000 dilution was 389, well below the expected 500 for a 10-fold difference in dilutions. This highlights the issue of inaccuracy when colony counts are greater than 300 and more than one bacterial cell grows into a single colony.



In the pour plate method of cell counting, the sample is mixed in liquid warm agar (45–50 °C) poured into a sterile Petri dish and further mixed by swirling. This process is repeated for each serial dilution prepared. The resulting colonies are counted and provide an estimate of the number of cells in the original volume sampled.

Spread Plate Method



In the spread plate method of cell counting, the sample is poured onto solid agar and then spread using a sterile spreader. This process is repeated for each serial dilution prepared. The resulting colonies are counted and provide an estimate of the number of cells in the original volume samples.

A very dilute sample—drinking water, for example—may not contain enough organisms to use either of the plate count methods described. In such cases, the original sample must be concentrated rather than diluted before plating. This can be accomplished using a modification of the plate count technique called the **membrane filtration technique**. Known volumes are vacuum-filtered aseptically through a membrane with a pore size small enough to trap microorganisms. The membrane is transferred to a Petri plate containing an appropriate growth medium. Colonies are counted after incubation. Calculation of the cell density is made by dividing the cell count by the volume of filtered liquid.

Note:



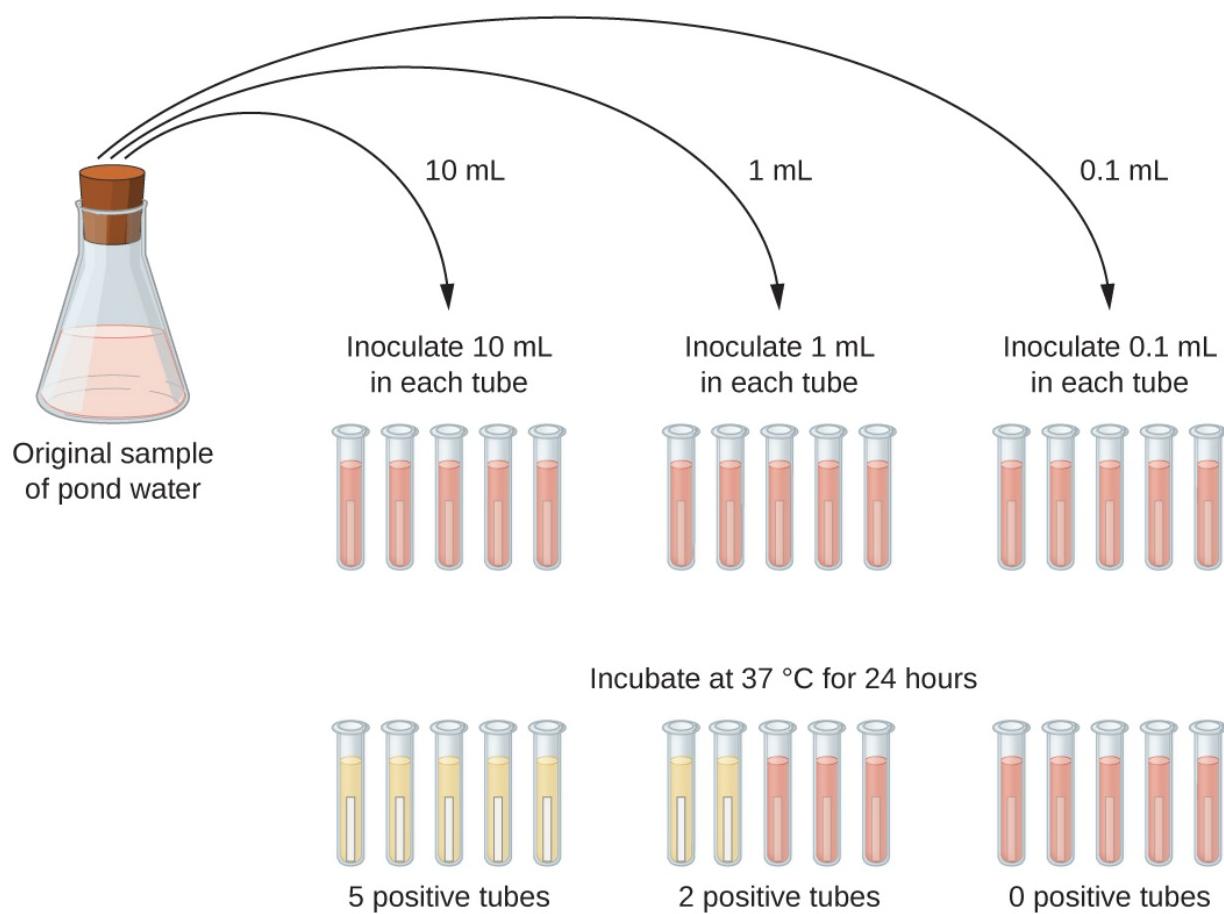
Watch this [video](#) for demonstrations of serial dilutions and spread plate techniques.

The Most Probable Number

The number of microorganisms in dilute samples is usually too low to be detected by the plate count methods described thus far. For these specimens, microbiologists routinely use the **most probable number (MPN) method**, a statistical procedure for estimating of the number of viable microorganisms in a sample. Often used for water and food samples, the MPN method evaluates detectable growth by observing changes in turbidity or color due to metabolic activity.

A typical application of MPN method is the estimation of the number of coliforms in a sample of pond water. Coliforms are gram-negative rod bacteria that ferment lactose. The presence of coliforms in water is considered a sign of contamination by fecal matter. For the method illustrated in [\[link\]](#), a series of three dilutions of the water sample is tested by inoculating five lactose broth tubes with 10 mL of sample, five lactose broth tubes with 1 mL of sample, and five lactose broth tubes with 0.1 mL of sample. The lactose broth tubes contain a pH indicator that changes color from red to yellow when the lactose is fermented. After inoculation and incubation, the tubes are examined for an indication of coliform growth by a color change in media from red to yellow. The first set of tubes (10-mL sample) showed growth in all the tubes; the second set of tubes (1 mL) showed growth in two tubes out of five; in the third set of tubes, no growth is observed in any of the tubes (0.1-mL dilution). The numbers 5, 2, and 0 are compared with [Figure B1](#) in [Appendix B](#), which has been constructed

using a probability model of the sampling procedure. From our reading of the table, we conclude that 49 is the most probable number of bacteria per 100 mL of pond water.no lo



In the most probable number method, sets of five lactose broth tubes are inoculated with three different volumes of pond water: 10 mL, 1 mL, and 0.1mL. Bacterial growth is assessed through a change in the color of the broth from red to yellow as lactose is fermented.

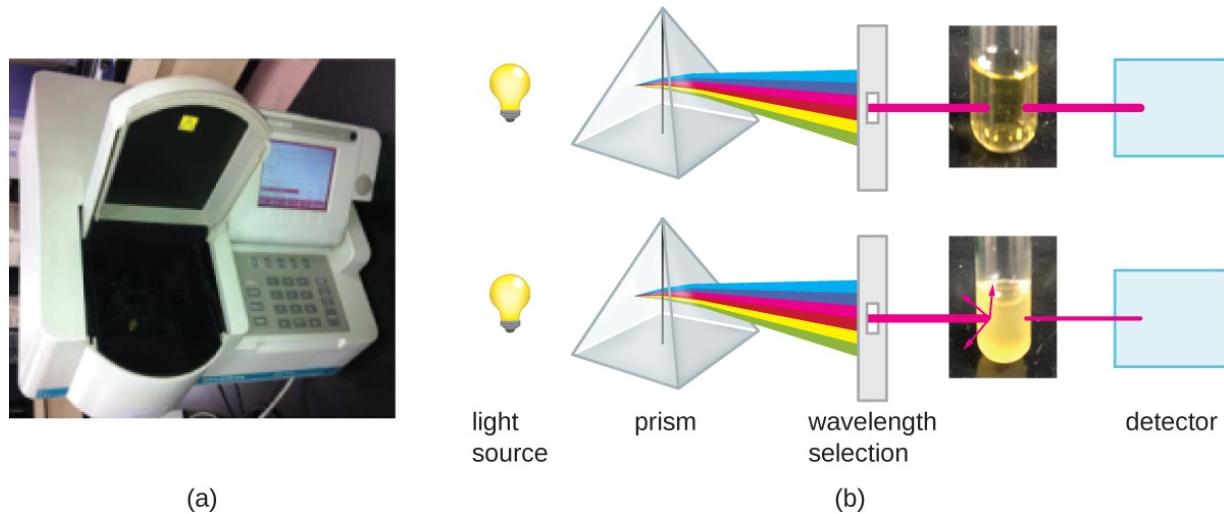
Note:

- What is a colony-forming unit?
- What two methods are frequently used to estimate bacterial numbers in water samples?

Indirect Cell Counts

Besides direct methods of counting cells, other methods, based on an indirect detection of cell density, are commonly used to estimate and compare cell densities in a culture. The foremost approach is to measure the **turbidity** (cloudiness) of a sample of bacteria in a liquid suspension. The laboratory instrument used to measure turbidity is called a spectrophotometer ([\[link\]](#)). In a spectrophotometer, a light beam is transmitted through a bacterial suspension, the light passing through the suspension is measured by a detector, and the amount of light passing through the sample and reaching the detector is converted to either percent transmission or a logarithmic value called absorbance (optical density). As the numbers of bacteria in a suspension increase, the turbidity also increases and causes less light to reach the detector. The decrease in light passing through the sample and reaching the detector is associated with a decrease in percent transmission and increase in absorbance measured by the spectrophotometer.

Measuring turbidity is a fast method to estimate cell density as long as there are enough cells in a sample to produce turbidity. It is possible to correlate turbidity readings to the actual number of cells by performing a viable plate count of samples taken from cultures having a range of absorbance values. Using these values, a calibration curve is generated by plotting turbidity as a function of cell density. Once the calibration curve has been produced, it can be used to estimate cell counts for all samples obtained or cultured under similar conditions and with densities within the range of values used to construct the curve.



(a) A spectrophotometer is commonly used to measure the turbidity of a bacterial cell suspension as an indirect measure of cell density. (b) A spectrophotometer works by splitting white light from a source into a spectrum. The spectrophotometer allows choice of the wavelength of light to use for the measurement. The optical density (turbidity) of the sample will depend on the wavelength, so once one wavelength is chosen, it must be used consistently. The filtered light passes through the sample (or a control with only medium) and the light intensity is measured by a detector. The light passing into a suspension of bacteria is scattered by the cells in such a way that some fraction of it never reaches the detector. This scattering happens to a far lesser degree in the control tube with only the medium. (credit a: modification of work by Hwang HS, Kim MS; credit b “test tube photos”: modification of work by Suzanne Wakim)

Measuring dry weight of a culture sample is another indirect method of evaluating culture density without directly measuring cell counts. The cell suspension used for weighing must be concentrated by filtration or centrifugation, washed, and then dried before the measurements are taken. The degree of drying must be standardized to account for residual water content. This method is especially useful for filamentous microorganisms, which are difficult to enumerate by direct or viable plate count.

As we have seen, methods to estimate viable cell numbers can be labor intensive and take time because cells must be grown. Recently, indirect ways of measuring live cells have been developed that are both fast and easy to implement. These methods measure cell activity by following the production of metabolic products or disappearance of reactants. Adenosine triphosphate (ATP) formation, biosynthesis of proteins and nucleic acids, and consumption of oxygen can all be monitored to estimate the number of cells.

Note:

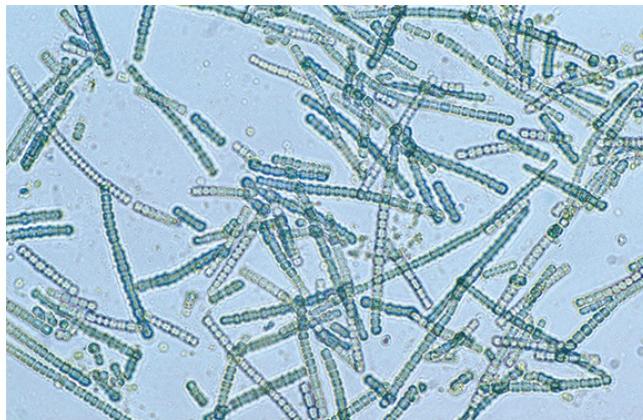
- What is the purpose of a calibration curve when estimating cell count from turbidity measurements?
- What are the newer indirect methods of counting live cells?

Alternative Patterns of Cell Division

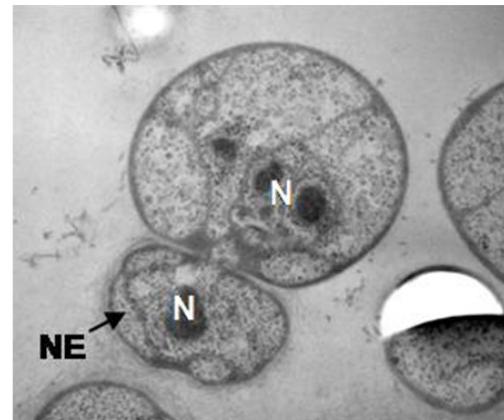
Binary fission is the most common pattern of cell division in prokaryotes, but it is not the only one. Other mechanisms usually involve asymmetrical division (as in budding) or production of spores in aerial filaments.

In some cyanobacteria, many nucleoids may accumulate in an enlarged round cell or along a filament, leading to the generation of many new cells at once. The new cells often split from the parent filament and float away in a process called **fragmentation** ([\[link\]](#)). Fragmentation is commonly observed in the Actinomycetes, a group of gram-positive, anaerobic bacteria commonly found in soil. Another curious example of cell division in prokaryotes, reminiscent of live birth in animals, is exhibited by the giant bacterium *Epulopiscium*. Several daughter cells grow fully in the parent cell, which eventually disintegrates, releasing the new cells to the environment. Other species may form a long narrow extension at one pole in a process called **budding**. The tip of the extension swells and forms a smaller cell, the bud that eventually detaches from the parent cell. Budding

is most common in yeast ([\[link\]](#)), but it is also observed in prosthecate bacteria and some cyanobacteria.



(a)



(b)

(a) Filamentous cyanobacteria, like those pictured here, replicate by fragmentation. (b) In this electron micrograph, cells of the bacterium *Gemmata obscuriglobus* are budding. The larger cell is the mother cell. Labels indicate the nucleoids (N) and the still-forming nuclear envelope (NE) of the daughter cell. (credit a: modification of work by CSIRO; credit b: modification of work by Kuo-Chang Lee, Rick I Webb and John A Fuerst)

The soil bacteria *Actinomyces* grow in long filaments divided by septa, similar to the mycelia seen in fungi, resulting in long cells with multiple nucleoids. Environmental signals, probably related to low nutrient availability, lead to the formation of aerial filaments. Within these aerial filaments, elongated cells divide simultaneously. The new cells, which contain a single nucleoid, develop into spores that give rise to new colonies.

Note:

- Identify at least one difference between fragmentation and budding.

Biofilms

In nature, microorganisms grow mainly in **biofilms**, complex and dynamic ecosystems that form on a variety of environmental surfaces, from industrial conduits and water treatment pipelines to rocks in river beds. Biofilms are not restricted to solid surface substrates, however. Almost any surface in a liquid environment containing some minimal nutrients will eventually develop a biofilm. Microbial mats that float on water, for example, are biofilms that contain large populations of photosynthetic microorganisms. Biofilms found in the human mouth may contain hundreds of bacterial species. Regardless of the environment where they occur, biofilms are not random collections of microorganisms; rather, they are highly structured communities that provide a selective advantage to their constituent microorganisms.

Biofilm Structure

Observations using confocal microscopy have shown that environmental conditions influence the overall structure of biofilms. Filamentous biofilms called streamers form in rapidly flowing water, such as freshwater streams, eddies, and specially designed laboratory flow cells that replicate growth conditions in fast-moving fluids. The streamers are anchored to the substrate by a “head” and the “tail” floats downstream in the current. In still or slow-moving water, biofilms mainly assume a mushroom-like shape. The structure of biofilms may also change with other environmental conditions such as nutrient availability.

Detailed observations of biofilms under confocal laser and scanning electron microscopes reveal clusters of microorganisms embedded in a matrix interspersed with open water channels. The extracellular matrix consists of **extracellular polymeric substances (EPS)** secreted by the

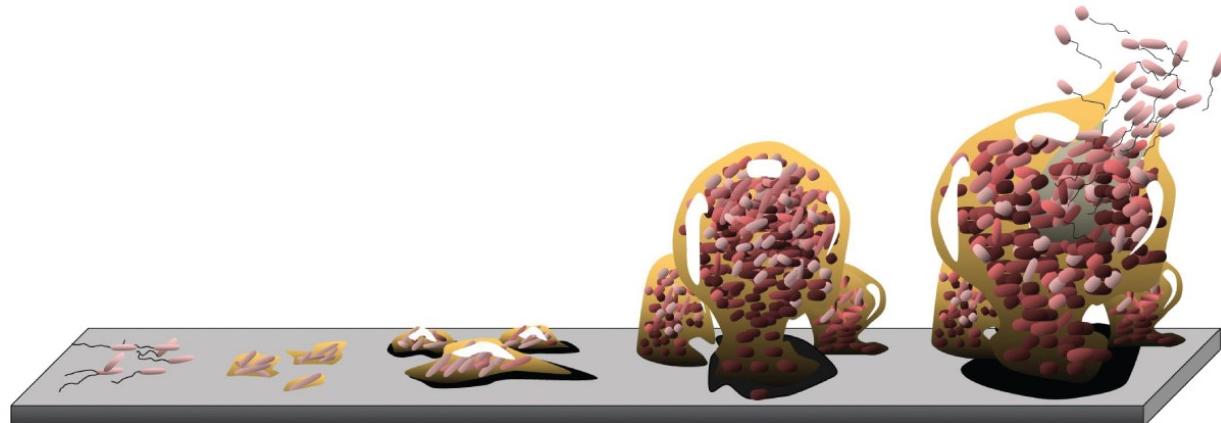
organisms in the biofilm. The extracellular matrix represents a large fraction of the biofilm, accounting for 50%–90% of the total dry mass. The properties of the EPS vary according to the resident organisms and environmental conditions.

EPS is a hydrated gel composed primarily of polysaccharides and containing other macromolecules such as proteins, nucleic acids, and lipids. It plays a key role in maintaining the integrity and function of the biofilm. Channels in the EPS allow movement of nutrients, waste, and gases throughout the biofilm. This keeps the cells hydrated, preventing desiccation. EPS also shelters organisms in the biofilm from predation by other microbes or cells (e.g., protozoans, white blood cells in the human body).

Biofilm Formation

Free-floating microbial cells that live in an aquatic environment are called **planktonic** cells. The formation of a biofilm essentially involves the attachment of planktonic cells to a substrate, where they become **sessile** (attached to a surface). This occurs in stages, as depicted in [\[link\]](#). The first stage involves the attachment of planktonic cells to a surface coated with a conditioning film of organic material. At this point, attachment to the substrate is reversible, but as cells express new phenotypes that facilitate the formation of EPS, they transition from a planktonic to a sessile lifestyle. The biofilm develops characteristic structures, including an extensive matrix and water channels. Appendages such as fimbriae, pili, and flagella interact with the EPS, and microscopy and genetic analysis suggest that such structures are required for the establishment of a mature biofilm. In the last stage of the biofilm life cycle, cells on the periphery of the biofilm revert to a planktonic lifestyle, sloughing off the mature biofilm to colonize new sites. This stage is referred to as dispersal.

- 1** Reversible attachment of planktonic cells. (seconds)
- 2** First colonizers become irreversibly attached. (second, minutes)
- 3** Growth and cell division. (hours, days)
- 4** Production of EPS and formation of water channels. (hours, days)
- 5** Attachment of secondary colonizers and dispersion of microbes to new sites. (days, months)



Stages in the formation and life cycle of a biofilm. (credit: modification of work by Public Library of Science and American Society for Microbiology)

Within a biofilm, different species of microorganisms establish metabolic collaborations in which the waste product of one organism becomes the nutrient for another. For example, aerobic microorganisms consume oxygen, creating anaerobic regions that promote the growth of anaerobes. This occurs in many polymicrobial infections that involve both aerobic and anaerobic pathogens.

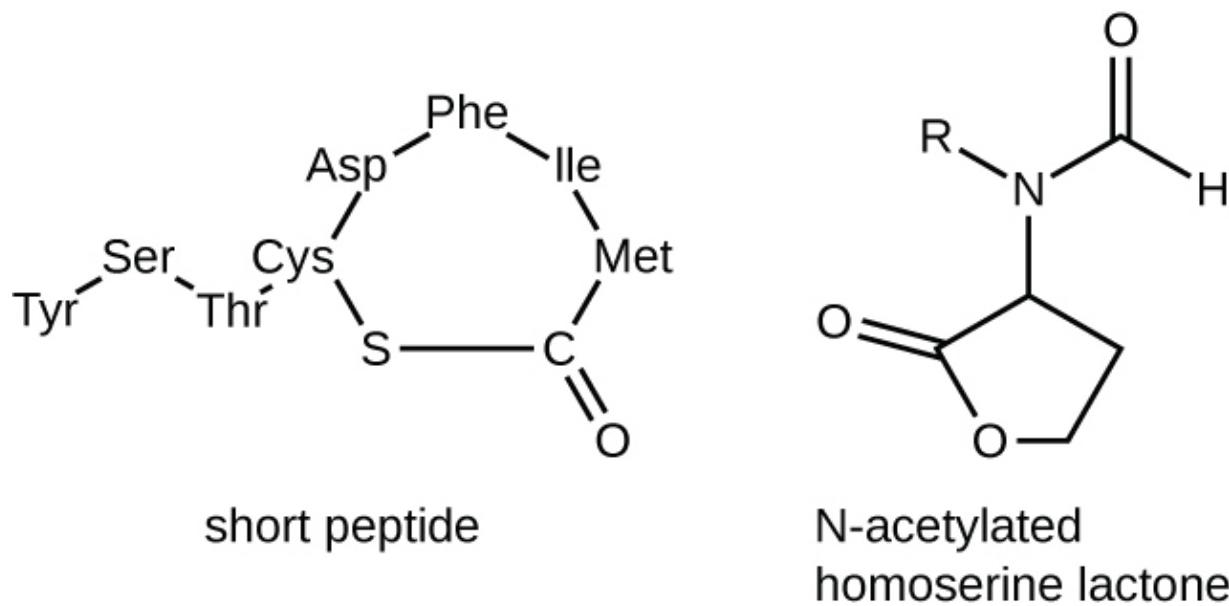
The mechanism by which cells in a biofilm coordinate their activities in response to environmental stimuli is called **quorum sensing**. Quorum sensing—which can occur between cells of different species within a biofilm—enables microorganisms to detect their cell density through the release and binding of small, diffusible molecules called **autoinducers**. When the cell population reaches a critical threshold (a quorum), these autoinducers initiate a cascade of reactions that activate genes associated with cellular functions that are beneficial only when the population reaches

a critical density. For example, in some pathogens, synthesis of virulence factors only begins when enough cells are present to overwhelm the immune defenses of the host. Although mostly studied in bacterial populations, quorum sensing takes place between bacteria and eukaryotes and between eukaryotic cells such as the fungus *Candida albicans*, a common member of the human microbiota that can cause infections in immunocompromised individuals. Other examples of possible outcomes due to quorum sensing are given on the table below .

Quorum sensing signaling molecule	Organism where the molecule is produced	Function regulated	Ecological importance
N-acylhomoserine lactone (AHL)	<i>Pseudomonas aeruginosa</i>	Biofilm formation and expression of virulence factors	Causes pneumonia on immunocompromised people
Hydroxy-palmitic acid methyl ester (PAME)	<i>Ralstonia solanacearum</i>	Increases virulence	Causes wilt in Irish potatoes, tomatoes, eggplants and several other plants
CAI-1	<i>Vibrio cholera</i>	Increases virulence and biofilm formation	Causes the disease cholera
Diffusible signal factor (DSF)-dependent QS system	<i>Xanthomonas campestris</i>	Protects against plants defenses and increases virulence	Causes wilt and black rot in cruciferous plants.

Examples of autoinducers involved in quorum sensing and their importance

The signaling molecules in quorum sensing belong to two major classes. Gram-negative bacteria communicate mainly using N-acylated homoserine lactones, whereas gram-positive bacteria mostly use small peptides ([\[link\]](#)). In all cases, the first step in quorum sensing consists of the binding of the autoinducer to its specific receptor only when a threshold concentration of signaling molecules is reached. Once binding to the receptor takes place, a cascade of signaling events leads to changes in gene expression. The result is the activation of biological responses linked to quorum sensing, notably an increase in the production of signaling molecules themselves, hence the term autoinducer.



Short peptides in gram-positive bacteria and N-acetylated homoserine lactones in gram-negative bacteria act as autoinducers in quorum sensing and mediate the coordinated response of bacterial cells. The R side chain of the N-acetylated homoserine lactone is specific for the species of gram-negative bacteria. Some secreted homoserine lactones are recognized by more than one species.

Biofilms and Human Health

The human body harbors many types of biofilms, some beneficial and some harmful. For example, the layers of normal microbiota lining the intestinal and respiratory mucosa play a role in warding off infections by pathogens. However, other biofilms in the body can have a detrimental effect on health. For example, the plaque that forms on teeth is a biofilm that can contribute to dental and periodontal disease. Biofilms can also form in wounds, sometimes causing serious infections that can spread. The bacterium *Pseudomonas aeruginosa* often colonizes biofilms in the airways of patients with cystic fibrosis, causing chronic and sometimes fatal infections of the lungs. Biofilms can also form on medical devices used in or on the body, causing infections in patients with in-dwelling catheters, artificial joints, or contact lenses.

Pathogens embedded within biofilms exhibit a higher resistance to antibiotics than their free-floating counterparts. Several hypotheses have been proposed to explain why. Cells in the deep layers of a biofilm are metabolically inactive and may be less susceptible to the action of antibiotics that disrupt metabolic activities. The EPS may also slow the diffusion of antibiotics and antiseptics, preventing them from reaching cells in the deeper layers of the biofilm. Phenotypic changes may also contribute to the increased resistance exhibited by bacterial cells in biofilms. For example, the increased production of efflux pumps, membrane-embedded proteins that actively extrude antibiotics out of bacterial cells, have been shown to be an important mechanism of antibiotic resistance among biofilm-associated bacteria. Finally, biofilms provide an ideal environment for the exchange of extrachromosomal DNA, which often includes genes that confer antibiotic resistance.

Note:

- What is the matrix of a biofilm composed of?
- What is the role of quorum sensing in a biofilm?

Key Concepts and Summary

- Most bacterial cells divide by **binary fission**. **Generation time** in bacterial growth is defined as the **doubling time** of the population.
- Cells in a closed system follow a pattern of growth with four phases: **lag, logarithmic (exponential), stationary, and death**.
- Cells can be counted by **direct viable cell count**. The **pour plate** and **spread plate** methods are used to plate **serial dilutions** into or onto, respectively, agar to allow counting of viable cells that give rise to **colony-forming units**. **Membrane filtration** is used to count live cells in dilute solutions. The **most probable cell number (MPN)** method allows estimation of cell numbers in cultures without using solid media.
- Indirect methods can be used to estimate **culture density** by measuring **turbidity** of a culture or live cell density by measuring metabolic activity.
- Other patterns of cell division include multiple nucleoid formation in cells; asymmetric division, as in **budding**; and the formation of hyphae and terminal spores.
- **Biofilms** are communities of microorganisms enmeshed in a matrix of **extracellular polymeric substance**. The formation of a biofilm occurs when **planktonic** cells attach to a substrate and become **sessile**. Cells in biofilms coordinate their activity by communicating through **quorum sensing**.
- Biofilms are commonly found on surfaces in nature and in the human body, where they may be beneficial or cause severe infections. Pathogens associated with biofilms are often more resistant to antibiotics and disinfectants.

Short Answer

Exercise:

Problem:

Why is it important to measure the transmission of light through a control tube with only broth in it when making turbidity measures of bacterial cultures?

Exercise:**Problem:**

In terms of counting cells, what does a plating method accomplish that an electronic cell counting method does not?

Exercise:**Problem:**

Order the following stages of the development of a biofilm from the earliest to the last step.

1. secretion of EPS
2. reversible attachment
3. dispersal
4. formation of water channels
5. irreversible attachment

Exercise:**Problem:**

Infections among hospitalized patients are often related to the presence of a medical device in the patient. Which conditions favor the formation of biofilms on in-dwelling catheters and prostheses?

Critical Thinking

Exercise:

Problem: Why are autoinducers small molecules?

Exercise:

Problem:

Refer to [Figure B1](#) in [Appendix B](#). If the results from a pond water sample were recorded as 3, 2, 1, what would be the MPN of bacteria in 100 mL of pond water?

Exercise:

Problem:

Refer to [\[link\]](#). Why does turbidity lose reliability at high cell concentrations when the culture reaches the stationary phase?

Oxygen Requirements for Microbial Growth

LEARNING OBJECTIVES

- Interpret visual data demonstrating minimum, optimum, and maximum oxygen or carbon dioxide requirements for growth
- Identify and describe different categories of microbes with requirements for growth with or without oxygen: obligate aerobe, obligate anaerobe, facultative anaerobe, aerotolerant anaerobe, microaerophile, and capnophile
- Give examples of microorganisms for each category of growth requirements

Ask most people “What are the major requirements for life?” and the answers are likely to include water and oxygen. Few would argue about the need for water, but what about oxygen? Can there be life without oxygen?

The answer is that molecular oxygen (O_2) is not always needed. The earliest signs of life are dated to a period when conditions on earth were highly reducing and free oxygen gas was essentially nonexistent. Only after cyanobacteria started releasing oxygen as a byproduct of photosynthesis and the capacity of iron in the oceans for taking up oxygen was exhausted did oxygen levels increase in the atmosphere. This event, often referred to as the Great Oxygenation Event or the Oxygen Revolution, caused a massive extinction. Most organisms could not survive the powerful oxidative properties of **reactive oxygen species** (ROS), highly unstable ions and molecules derived from partial reduction of oxygen that can damage virtually any macromolecule or structure with which they come in contact. Singlet oxygen ($O_2\bullet$), superoxide (O_2^-), peroxides (H_2O_2), hydroxyl

radical ($\text{OH}\cdot$), and hypochlorite ion (OCl^-), the active ingredient of household bleach, are all examples of ROS. The organisms that were able to detoxify reactive oxygen species harnessed the high electronegativity of oxygen to produce free energy for their metabolism and thrived in the new environment.

Oxygen Requirements of Microorganisms

Many ecosystems are still free of molecular oxygen. Some are found in extreme locations, such as deep in the ocean or in earth's crust; others are part of our everyday landscape, such as marshes, bogs, and sewers. Within the bodies of humans and other animals, regions with little or no oxygen provide an anaerobic environment for microorganisms. ([\[link\]](#)).



(a)



(b)

Anaerobic environments are still common on earth. They include environments like (a) a bog where undisturbed dense sediments are virtually devoid of oxygen, and (b) the rumen (the first compartment of a cow's stomach), which provides an oxygen-free incubator for methanogens and other obligate anaerobic bacteria. (credit a: modification of work by National Park Service; credit b: modification of work by US Department of Agriculture)

We can easily observe different requirements for molecular oxygen by growing bacteria in **thioglycolate tube cultures**. A test-tube culture starts with autoclaved **thioglycolate medium** containing a low percentage of agar to allow motile bacteria to move throughout the medium. Thioglycolate has strong reducing properties and autoclaving flushes out most of the oxygen. The tubes are inoculated with the bacterial cultures to be tested and incubated at an appropriate temperature. Over time, oxygen slowly diffuses throughout the thioglycolate tube culture from the top. Bacterial density increases in the area where oxygen concentration is best suited for the growth of that particular organism.

The growth of bacteria with varying oxygen requirements in thioglycolate tubes is illustrated in [\[link\]](#). In tube A, all the growth is seen at the top of the tube. The bacteria are **obligate (strict) aerobes** that cannot grow without an abundant supply of oxygen. Tube B looks like the opposite of tube A. Bacteria grow at the bottom of tube B. Those are **obligate anaerobes**, which are killed by oxygen. Tube C shows heavy growth at the top of the tube and growth throughout the tube, a typical result with **facultative anaerobes**. Facultative anaerobes are organisms that thrive in the presence of oxygen but also grow in its absence by relying on fermentation or anaerobic respiration, if there is a suitable electron acceptor other than oxygen and the organism is able to perform anaerobic respiration. The **aerotolerant anaerobes** in tube D are indifferent to the presence of oxygen. They do not use oxygen because they usually have a fermentative metabolism, but they are not harmed by the presence of oxygen as obligate anaerobes are. Tube E on the right shows a “Goldilocks” culture. The oxygen level has to be just right for growth, not too much and not too little. These **microaerophiles** are bacteria that require a minimum level of oxygen for growth, about 1%–10%, well below the 21% found in the atmosphere.

Examples of obligate aerobes are *Mycobacterium tuberculosis*, the causative agent of tuberculosis and *Micrococcus luteus*, a gram-positive bacterium that colonizes the skin. *Neisseria meningitidis*, the causative agent of severe bacterial meningitis, and *N. gonorrhoeae*, the causative agent of sexually transmitted gonorrhea, are also obligate aerobes.

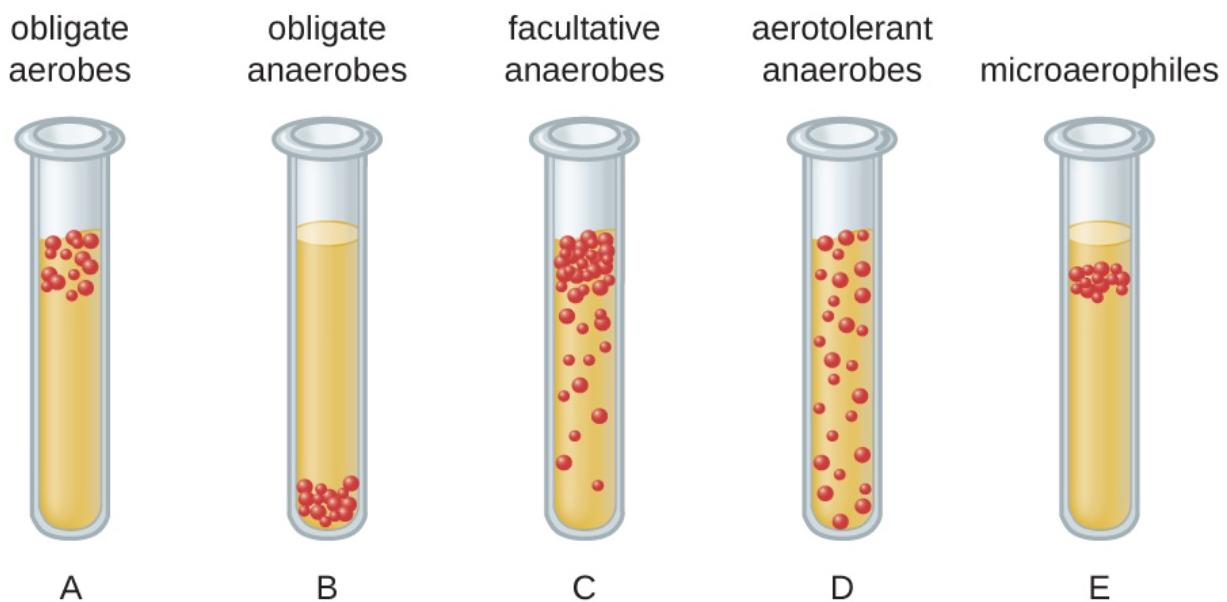


Diagram of bacterial cell distribution in thioglycolate tubes.

Many obligate anaerobes are found in the environment where anaerobic conditions exist, such as in deep sediments of soil, still waters, and at the bottom of the deep ocean where there is no photosynthetic life. Anaerobic conditions also exist naturally in the intestinal tract of animals. Obligate anaerobes, mainly *Bacteroidetes*, represent a large fraction of the microbes in the human gut. Transient anaerobic conditions exist when tissues are not supplied with blood circulation; they die and become an ideal breeding ground for obligate anaerobes. Another type of obligate anaerobe encountered in the human body is the gram-positive, rod-shaped *Clostridium* spp. Their ability to form endospores allows them to survive in the presence of oxygen. One of the major causes of health-acquired infections is *C. difficile*, known as C. diff. Prolonged use of antibiotics for other infections increases the probability of a patient developing a secondary *C. difficile* infection. Antibiotic treatment disrupts the balance of microorganisms in the intestine and allows the colonization of the gut by *C. difficile*, causing a significant inflammation of the colon.

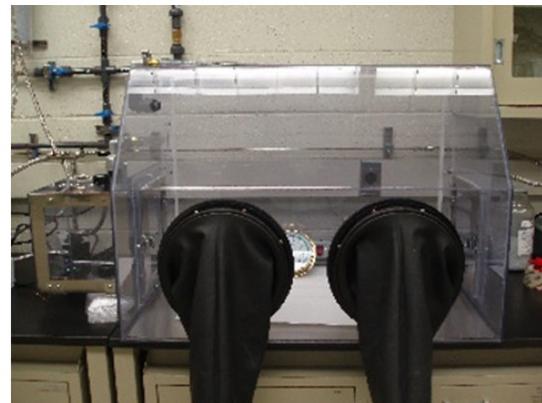
Other clostridia responsible for serious infections include *C. tetani*, the agent of tetanus, and *C. perfringens*, which causes gas gangrene. In both

cases, the infection starts in necrotic tissue (dead tissue that is not supplied with oxygen by blood circulation). This is the reason that deep puncture wounds are associated with tetanus. When tissue death is accompanied by lack of circulation, gangrene is always a danger.

The study of obligate anaerobes requires special equipment. Obligate anaerobic bacteria must be grown under conditions devoid of oxygen. The most common approach is culture in an **anaerobic jar** ([\[link\]](#)). Anaerobic jars include chemical packs that remove oxygen and release carbon dioxide (CO₂). An **anaerobic chamber** is an enclosed box from which all oxygen is removed. Gloves sealed to openings in the box allow handling of the cultures without exposing the culture to air ([\[link\]](#)).



(a)



(b)

(a) An anaerobic jar is pictured that is holding nine Petri plates supporting cultures. (b) Openings in the side of an anaerobic box are sealed by glove-like sleeves that allow for the handling of cultures inside the box. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by NIST)

Staphylococci and Enterobacteriaceae are examples of facultative anaerobes. Staphylococci are found on the skin and upper respiratory tract. Enterobacteriaceae are found primarily in the gut and upper respiratory tract

but can sometimes spread to the urinary tract, where they are capable of causing infections. It is not unusual to see mixed bacterial infections in which the facultative anaerobes use up the oxygen, creating an environment for the obligate anaerobes to flourish.

Examples of aerotolerant anaerobes include lactobacilli and streptococci, both found in the oral microbiota. *Campylobacter jejuni*, which causes gastrointestinal infections, is an example of a microaerophile and is grown under low-oxygen conditions.

The **optimum oxygen concentration**, as the name implies, is the ideal concentration of oxygen for a particular microorganism. The lowest concentration of oxygen that allows growth is called the **minimum permissive oxygen concentration**. The highest tolerated concentration of oxygen is the **maximum permissive oxygen concentration**. The organism will not grow outside the range of oxygen levels found between the minimum and maximum permissive oxygen concentrations.

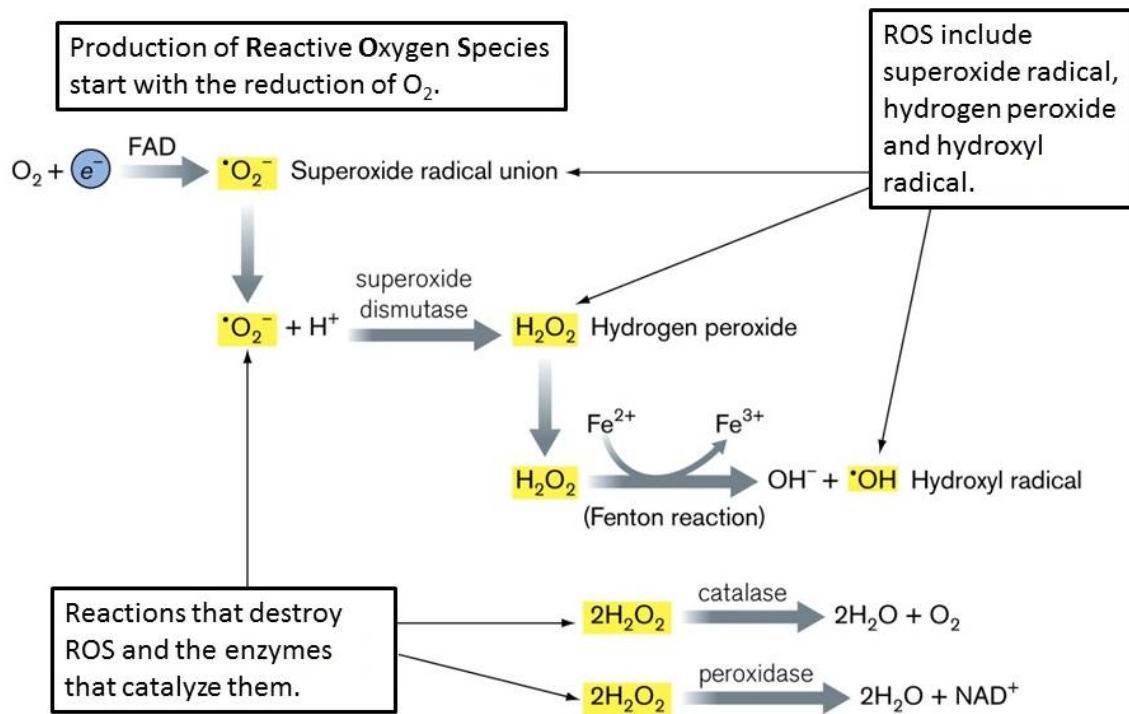
Note:

- Would you expect the oldest bacterial lineages to be aerobic or anaerobic?
- Which bacteria grow at the top of a thioglycolate tube, and which grow at the bottom of the tube?

Detoxification of Reactive Oxygen Species

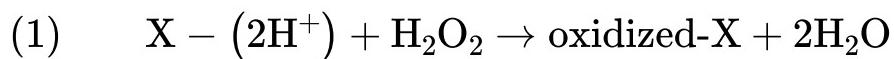
Aerobic respiration constantly generates reactive oxygen species (ROS), byproducts that must be detoxified. Even organisms that do not use aerobic respiration need some way to break down some of the ROS that may form from atmospheric oxygen. Three main enzymes break down those toxic byproducts: superoxide dismutase, peroxidase, and catalase. Each one

catalyzes a different reaction. Reactions of type seen in Reaction 1 are catalyzed by **peroxidases**.



Reactive oxygen species such as superoxide radical, hydrogen peroxide, and hydroxyl radical can damage nucleic acids, proteins, and lipids

Equation:



In these reactions, an electron donor (reduced compound; e.g., reduced nicotinamide adenine dinucleotide [NADH]) oxidizes hydrogen peroxide,

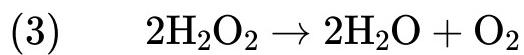
or other peroxides, to water. The enzymes play an important role by limiting the damage caused by peroxidation of membrane lipids. Reaction 2 is mediated by the enzyme **superoxide dismutase** (SOD) and breaks down the powerful superoxide anions generated by aerobic metabolism:

Equation:

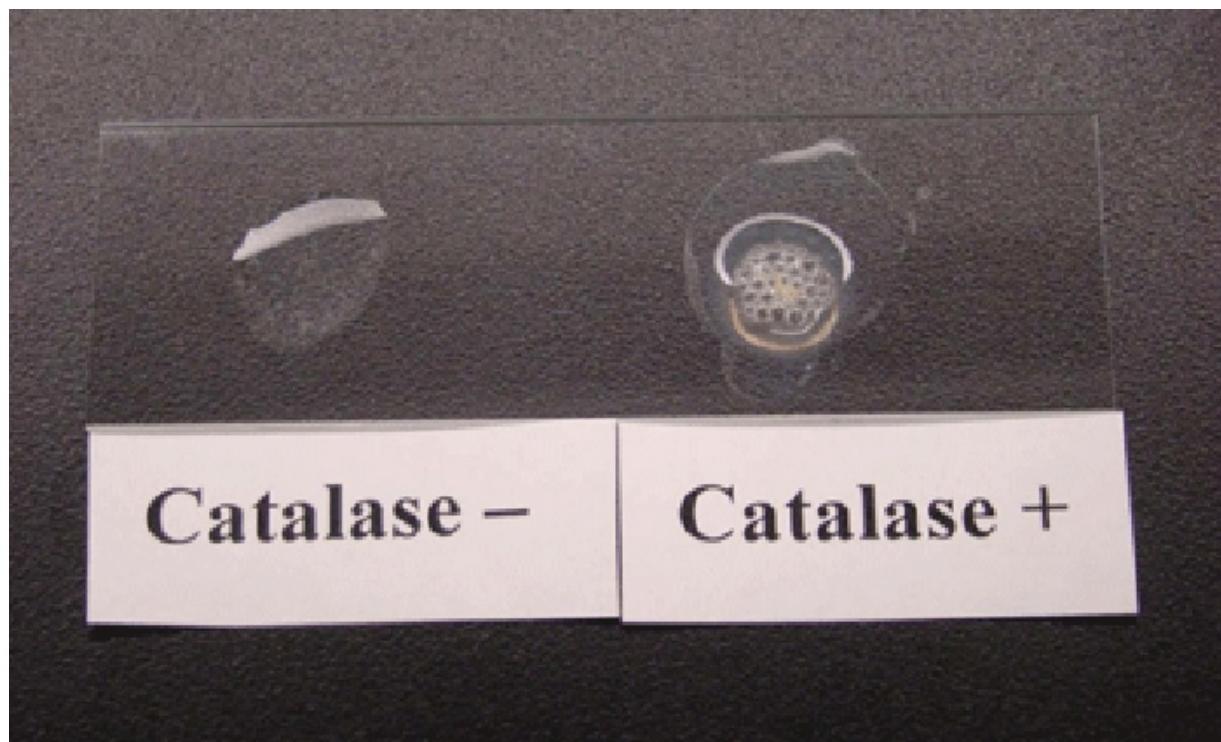


The enzyme **catalase** converts hydrogen peroxide to water and oxygen as shown in Reaction 3.

Equation:



Obligate anaerobes usually lack all three enzymes. Aerotolerant anaerobes do have SOD but no catalase. Reaction 3, shown occurring in [\[link\]](#), is the basis of a useful and rapid test to distinguish streptococci, which are aerotolerant and do not possess catalase, from staphylococci, which are facultative anaerobes. A sample of culture rapidly mixed in a drop of 3% hydrogen peroxide will release bubbles if the culture is catalase positive.



The catalase test detects the presence of the enzyme catalase by noting whether bubbles are released when hydrogen peroxide is added to a culture sample. Compare the positive result (right) with the negative result (left). (credit: Centers for Disease Control and Prevention)

Bacteria that grow best in a higher concentration of CO₂ and a lower concentration of oxygen than present in the atmosphere are called **capnophiles**. One common approach to grow capnophiles is to use a **candle jar**. A candle jar consists of a jar with a tight-fitting lid that can accommodate the cultures and a candle. After the cultures are added to the jar, the candle is lit and the lid closed. As the candle burns, it consumes most of the oxygen present and releases CO₂.

Note:

- What substance is added to a sample to detect catalase?

- What is the function of the candle in a candle jar?

Key Concepts and Summary

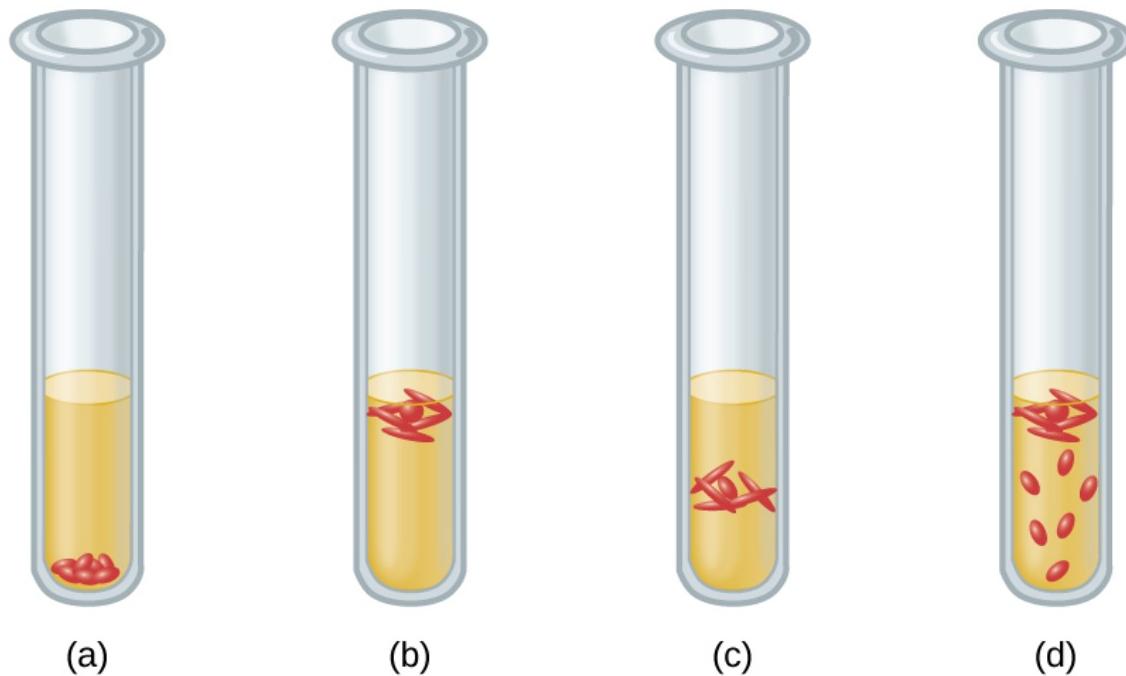
- Aerobic and anaerobic environments can be found in diverse niches throughout nature, including different sites within and on the human body.
- Microorganisms vary in their requirements for molecular oxygen.
Obligate aerobes depend on aerobic respiration and use oxygen as a terminal electron acceptor. They cannot grow without oxygen.
- **Obligate anaerobes** cannot grow in the presence of oxygen. They depend on fermentation and anaerobic respiration using a final electron acceptor other than oxygen.
- **Facultative anaerobes** show better growth in the presence of oxygen but will also grow without it.
- Although **aerotolerant anaerobes** do not perform aerobic respiration, they can grow in the presence of oxygen. Most aerotolerant anaerobes test negative for the enzyme **catalase**.
- **Microaerophiles** need oxygen to grow, albeit at a lower concentration than 21% oxygen in air.
- **Optimum oxygen concentration** for an organism is the oxygen level that promotes the fastest growth rate. The **minimum permissive oxygen concentration** and the **maximum permissive oxygen concentration** are, respectively, the lowest and the highest oxygen levels that the organism will tolerate.
- **Peroxidase**, **superoxide dismutase**, and **catalase** are the main enzymes involved in the detoxification of the **reactive oxygen species**. Superoxide dismutase is usually present in a cell that can tolerate oxygen. All three enzymes are usually detectable in cells that perform aerobic respiration and produce more ROS.
- A **capnophile** is an organism that requires a higher than atmospheric concentration of CO₂ to grow.

Matching

Exercise:

Problem:

Four tubes are illustrated with cultures grown in a medium that slows oxygen diffusion. Match the culture tube with the correct type of bacteria from the following list: facultative anaerobe, obligate anaerobe, microaerophile, aerotolerant anaerobe, obligate aerobe.



Solution:

(a) obligate anaerobe, (b) obligate aerobe, (c) microaerophile, (d) facultative anaerobe

Short Answer

Exercise:

Problem:

Why are some obligate anaerobes able to grow in tissues (e.g., gum pockets) that are not completely free of oxygen?

Exercise:**Problem:**

Why should *Haemophilus influenzae* be grown in a candle jar?

Exercise:**Problem:**

In terms of oxygen requirements, what type of organism would most likely be responsible for a foodborne illness associated with canned foods?

Critical Thinking

Exercise:**Problem:**

A microbiology instructor prepares cultures for a gram-staining practical laboratory by inoculating growth medium with a gram-positive coccus (nonmotile) and a gram-negative rod (motile). The goal is to demonstrate staining of a mixed culture. The flask is incubated at 35 °C for 24 hours without aeration. A sample is stained and reveals only gram-negative rods. Both cultures are known facultative anaerobes. Give a likely reason for success of the gram-negative rod. Assume that the cultures have comparable intrinsic growth rates.

The Effects of pH on Microbial Growth

LEARNING OBJECTIVES

- Illustrate and briefly describe minimum, optimum, and maximum pH requirements for growth
- Identify and describe the different categories of microbes with pH requirements for growth: acidophiles, neutrophiles, and alkaliphiles
- Discuss the importance of keeping pH homeostasis in microbial cells.
- Explain how acidophiles, neutrophiles, and alkaliphiles maintain their pH homeostasis.

Yogurt, pickles, sauerkraut, and lime-seasoned dishes all owe their tangy taste to a high acid content ([\[link\]](#)). Recall that acidity is a function of the concentration of hydrogen ions $[H^+]$ and is measured as pH. Environments with pH values below 7.0 are considered acidic, whereas those with pH values above 7.0 are considered basic. Extreme pH affects the structure of all macromolecules. The hydrogen bonds holding together strands of DNA break up at high pH. Lipids are hydrolyzed by an extremely basic pH. The proton motive force responsible for production of ATP in cellular respiration depends on the concentration gradient of H^+ across the plasma membrane (see [Cellular Respiration](#)). If H^+ ions are neutralized by hydroxide ions, the concentration gradient collapses and impairs energy production. But the component most sensitive to pH in the cell is its workhorse, the protein. Moderate changes in pH modify the ionization of amino-acid functional groups and disrupt hydrogen bonding, which, in turn,

promotes changes in the folding of the molecule, promoting denaturation and destroying activity.



Lactic acid bacteria that ferment milk into yogurt or transform vegetables in pickles thrive at a pH close to 4.0. Sauerkraut and dishes such as pico de gallo owe their tangy flavor to their acidity. Acidic foods have been a mainstay of the human diet for centuries, partly because most microbes that cause food spoilage grow best at a near neutral pH and do not tolerate acidity well. (credit “yogurt”: modification of work by “nina.jsp”/Flickr; credit “pickles”: modification of work by Noah Sussman; credit “sauerkraut”: modification of work by Jesse LaBuff; credit “pico de gallo”: modification of work by “regan76”/Flickr)

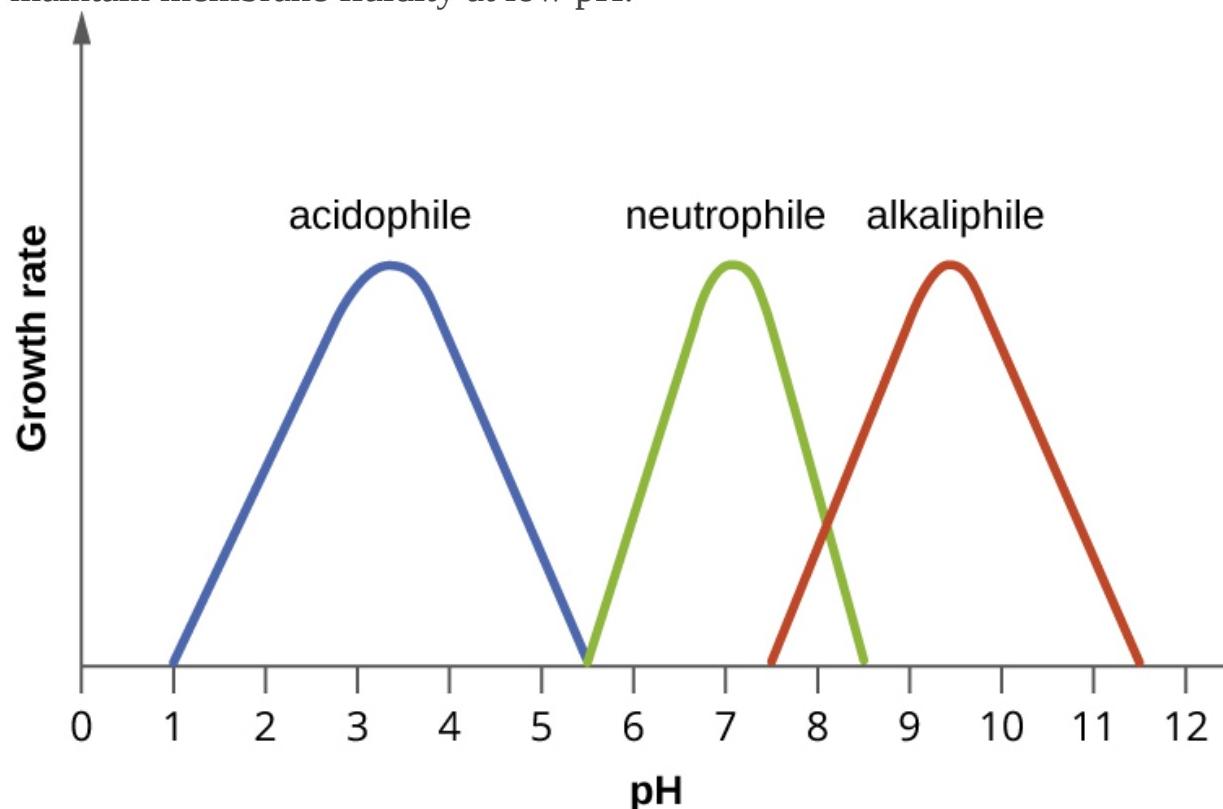
The **optimum growth pH** is the most favorable pH for the growth of an organism. The lowest pH value that an organism can tolerate is called the **minimum growth pH** and the highest pH is the **maximum growth pH**. These values can cover a wide range, which is important for the preservation of food and to microorganisms' survival in the stomach. For example, the optimum growth pH of *Salmonella* spp. is 7.0–7.5, but the minimum growth pH is closer to 4.2.

Most bacteria are **neutrophiles**, meaning they grow optimally at a pH within one or two pH units of the neutral pH of 7 (see [[link](#)]), maintaining a pH difference across the membrane. This pH difference is an important component of the proton motive force, a source of energy for the cell. Most familiar bacteria, like *Escherichia coli*, staphylococci, and *Salmonella* spp. are neutrophiles and do not fare well in the acidic pH of the stomach. However, there are pathogenic strains of *E. coli*, *S. typhi*, and other species

of intestinal pathogens that are much more resistant to stomach acid. In comparison, fungi thrive at slightly acidic pH values of 5.0–6.0.

Microorganisms that grow optimally at pH less than 5.55 are called **acidophiles**. For example, the sulfur-oxidizing *Sulfolobus* spp. isolated from sulfur mud fields and hot springs in Yellowstone National Park are extreme acidophiles. These archaea survive at pH values of 2.5–3.5.

Species of the archaean genus *Ferroplasma* live in acid mine drainage at pH values of 0–2.9. *Lactobacillus* bacteria, which are an important part of the normal microbiota of the vagina, can tolerate acidic environments at pH values 3.5–6.8 and also contribute to the acidity of the vagina (pH of 4, except at the onset of menstruation) through their metabolic production of lactic acid. The vagina's acidity plays an important role in inhibiting other microbes that are less tolerant of acidity. Acidophilic microorganisms display a number of adaptations to survive in strong acidic environments. For example, proteins show increased negative surface charge that stabilizes them at low pH. Pumps actively eject H⁺ ions out of the cells. The changes in the composition of membrane phospholipids probably reflect the need to maintain membrane fluidity at low pH.



The curves show the approximate pH ranges for the growth of the different classes of pH-specific prokaryotes. Each curve has an optimal pH and extreme pH values at which growth is much reduced. Most bacteria are neutrophiles and grow best at near-neutral pH (center curve). Acidophiles have optimal growth at pH values near 3 and alkaliphiles have optimal growth at pH values above 9.

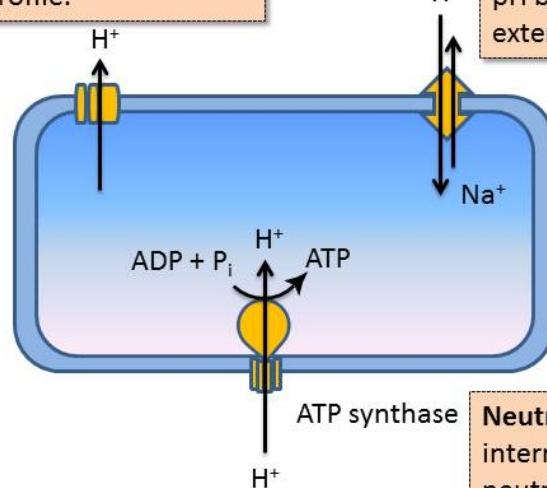
At the other end of the spectrum are **alkaliphiles**, microorganisms that grow best at pH between 8.0 and 10.5. *Vibrio cholerae*, the pathogenic agent of cholera, grows best at the slightly basic pH of 8.0; it can survive pH values of 11.0 but is inactivated by the acid of the stomach. When it comes to survival at high pH, the bright pink archaean *Natronobacterium*, found in the soda lakes of the African Rift Valley, may hold the record at a pH of 10.5 ([\[link\]](#)). Extreme alkaliphiles have adapted to their harsh environment through evolutionary modification of lipid and protein structure and compensatory mechanisms to maintain the proton motive force in an alkaline environment. For example, the alkaliphile *Bacillus firmus* derives the energy for transport reactions and motility from a Na^+ ion gradient rather than a proton motive force. Many enzymes from alkaliphiles have a higher isoelectric point, due to an increase in the number of basic amino acids, than homologous enzymes from neutrophiles.



View from space of Lake Natron in Tanzania. The pink color is due to the pigmentation of the extreme alkaliphilic and halophilic microbes that colonize the lake. (credit: NASA)

Besides of pumping H^+ out of the cell to keep a close to neutral internal environment, acidophiles show a decrease in H^+ membrane permeability due to altered lipid profile.

Alkaliphiles use Na^+/H^+ antiports to bring H^+ into the cell keeping the internal pH below the alkaline external environment.



Strategies applied by neutrophiles, alkaliphiles, and acidophiles to keep proton circulation and pH homeostasis.

Note:

Survival at the Low pH of the Stomach

Peptic ulcers (or stomach ulcers) are painful sores on the stomach lining. Until the 1980s, they were believed to be caused by spicy foods, stress, or a combination of both. Patients were typically advised to eat bland foods, take anti-acid medications, and avoid stress. These remedies were not particularly effective, and the condition often recurred. This all changed dramatically when the real cause of most peptic ulcers was discovered to be a slim, corkscrew-shaped bacterium, *Helicobacter pylori*. This organism

was identified and isolated by Barry Marshall and Robin Warren, whose discovery earned them the Nobel Prize in Medicine in 2005.

The ability of *H. pylori* to survive the low pH of the stomach would seem to suggest that it is an extreme acidophile. As it turns out, this is not the case. In fact, *H. pylori* is a neutrophile. So, how does it survive in the stomach? Remarkably, *H. pylori* creates a microenvironment in which the pH is nearly neutral. It achieves this by producing large amounts of the enzyme urease, which breaks down urea to form NH_4^+ and CO_2 . The ammonium ion raises the pH of the immediate environment.

This metabolic capability of *H. pylori* is the basis of an accurate, noninvasive test for infection. The patient is given a solution of urea containing radioactively labeled carbon atoms. If *H. pylori* is present in the stomach, it will rapidly break down the urea, producing radioactive CO_2 that can be detected in the patient's breath. Because peptic ulcers may lead to gastric cancer, patients who are determined to have *H. pylori* infections are treated with antibiotics.

Note:

- What effect do extremes of pH have on proteins?
- What pH-adaptive type of bacteria would most human pathogens be?

Key Concepts and Summary

- Bacteria are generally **neutrophiles**. They grow best at neutral pH close to 7.0.
- **Acidophiles** grow optimally at a pH near 3.0. **Alkaliphiles** are organisms that grow optimally between a pH of 8 and 10.5. Extreme acidophiles and alkaliphiles grow slowly or not at all near neutral pH.
- Microorganisms grow best at their **optimum growth pH**. Growth occurs slowly or not at all below the **minimum growth pH** and above the **maximum growth pH**.

Critical Thinking

Exercise:

Problem:

People who use proton pumps inhibitors or antacids are more prone to infections of the gastrointestinal tract. Can you explain the observation in light of what you have learned?

Temperature and Microbial Growth

LEARNING OBJECTIVES

- Illustrate and briefly describe minimum, optimum, and maximum temperature requirements for growth
- Identify and describe different categories of microbes with temperature requirements for growth: psychrophile, psychrotrophs, mesophile, thermophile, hyperthermophile
- Describe some of the adaptations microorganisms developed to survive in harsh environments

When the exploration of Lake Whillans started in Antarctica, researchers did not expect to find much life. Constant subzero temperatures and lack of obvious sources of nutrients did not seem to be conditions that would support a thriving ecosystem. To their surprise, the samples retrieved from the lake showed abundant microbial life. In a different but equally harsh setting, bacteria grow at the bottom of the ocean in sea vents ([\[link\]](#)), where temperatures can reach 340 °C (700 °F).

Microbes can be roughly classified according to the range of temperature at which they can grow. The growth rates are the highest at the **optimum growth temperature** for the organism. The lowest temperature at which the organism can survive and replicate is its **minimum growth temperature**. The highest temperature at which growth can occur is its **maximum growth temperature**. The following ranges of permissive growth temperatures are approximate only and can vary according to other environmental factors.

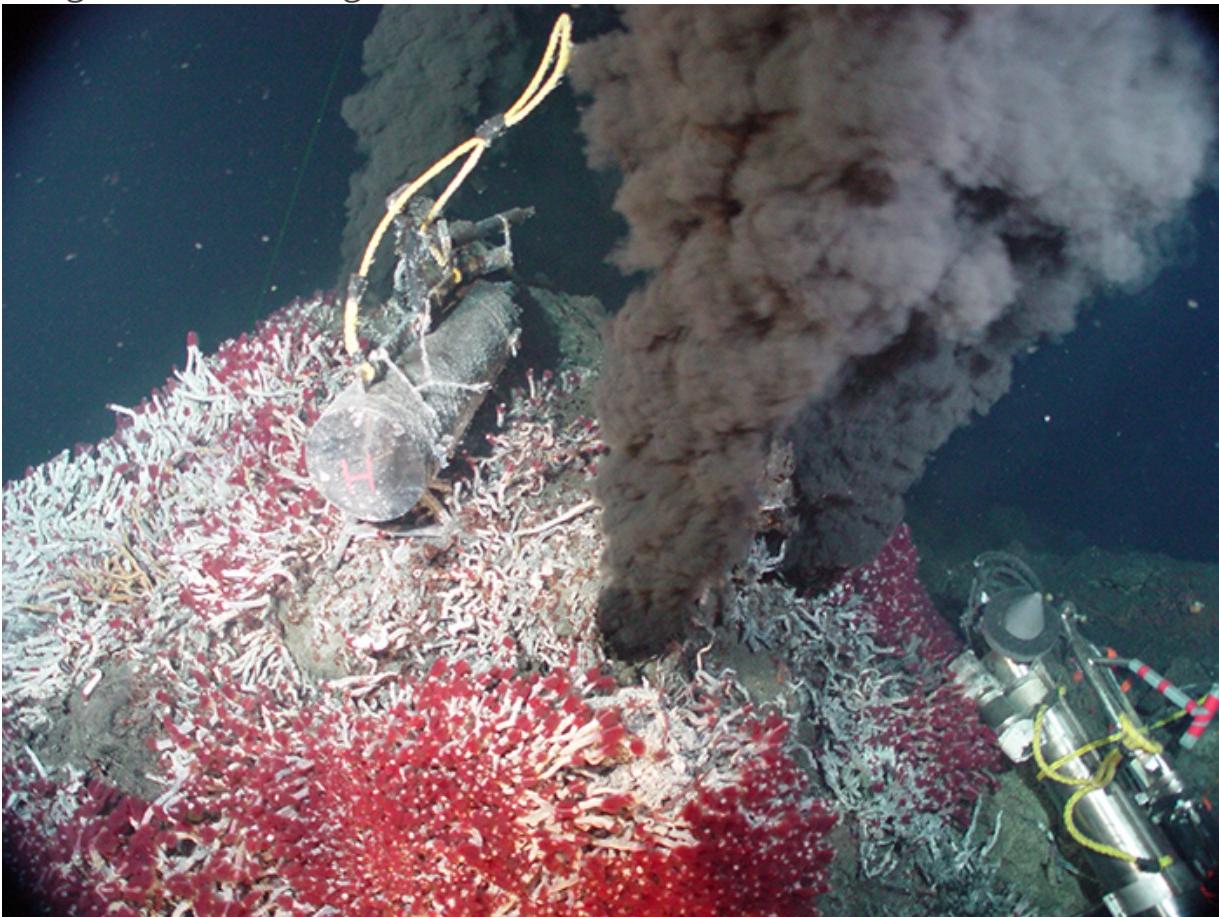
Organisms categorized as **mesophiles** (“middle loving”) are adapted to moderate temperatures, with optimal growth temperatures ranging from room temperature (about 20 °C) to about 45 °C. As would be expected from the core temperature of the human body, 37 °C (98.6 °F), normal human microbiota and pathogens (e.g., *E. coli*, *Salmonella* spp., and *Lactobacillus* spp.) are mesophiles.

Organisms called **psychrotrophs**, also known as psychrotolerant, prefer cooler environments, from a high temperature of 25 °C to refrigeration temperature about 4 °C. They are found in many natural environments in temperate climates. They are also responsible for the spoilage of refrigerated food.

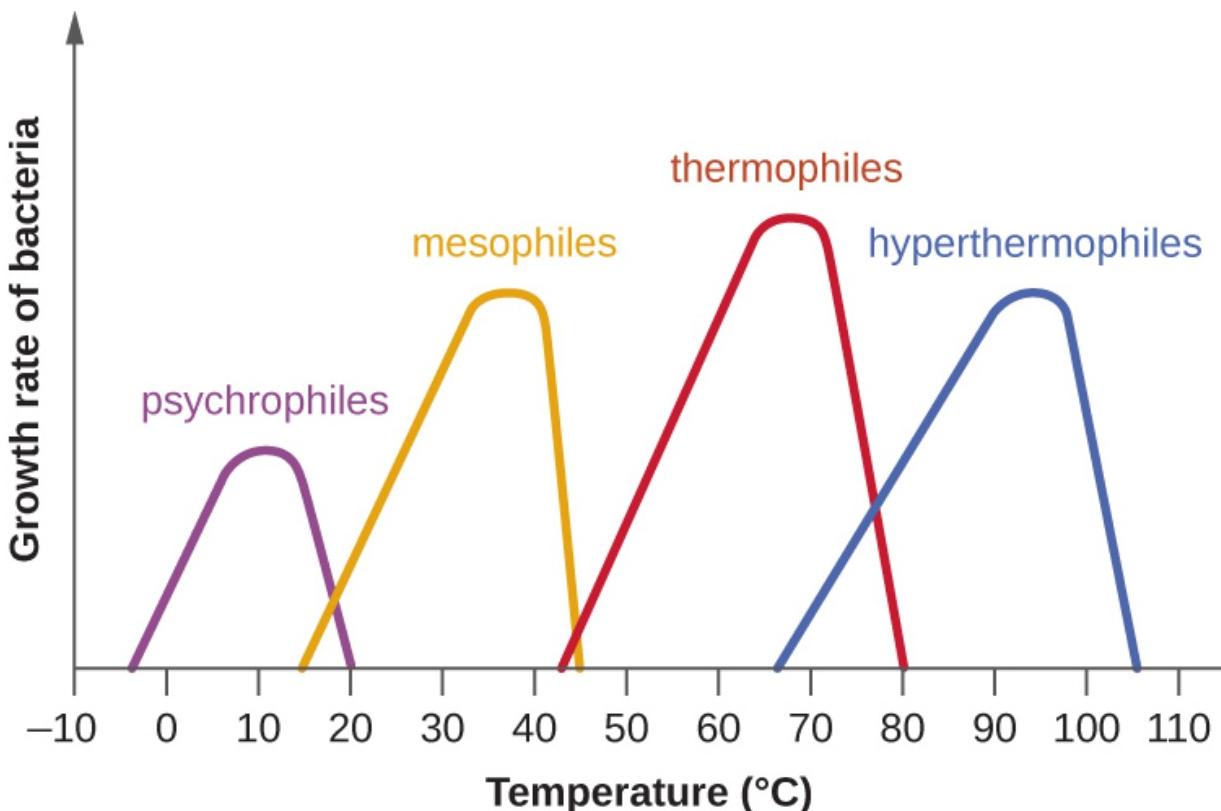
The organisms retrieved from arctic lakes such as Lake Whillans are considered extreme **psychrophiles** (cold loving). Psychrophiles are microorganisms that can grow at 0 °C and below, have an optimum growth temperature close to 15 °C, and usually do not survive at temperatures above 20 °C. They are found in permanently cold environments such as the deep waters of the oceans. Because they are active at low temperature, psychrophiles and psychrotrophs are important decomposers in cold climates.

Organisms that grow at optimum temperatures of 50 °C to a maximum of 80 °C are called **thermophiles** (“heat loving”). They do not multiply at room temperature. Thermophiles are widely distributed in hot springs, geothermal soils, and manmade environments such as garden compost piles where the microbes break down kitchen scraps and vegetal material. Examples of thermophiles include *Thermus aquaticus* and *Geobacillus* spp. Higher up on the extreme temperature scale we find the **hyperthermophiles**, which are characterized by growth ranges from 80 °C to a maximum of 110 °C, with some extreme examples that survive temperatures above 121 °C, the average temperature of an autoclave. The hydrothermal vents at the bottom of the ocean are a prime example of extreme environments, with temperatures reaching an estimated 340 °C ([\[link\]](#)). Microbes isolated from the vents achieve optimal growth at temperatures higher than 100 °C. Noteworthy examples are *Pyrobolus* and *Pyrodictium*, archaea that grow at 105 °C and survive autoclaving. [\[link\]](#)

shows the typical skewed curves of temperature-dependent growth for the categories of microorganisms we have discussed.



A black smoker at the bottom of the ocean belches hot, chemical-rich water, and heats the surrounding waters. Sea vents provide an extreme environment that is nonetheless teeming with macroscopic life (the red tubeworms) supported by an abundant microbial ecosystem. (credit: NOAA)



The graph shows growth rate of bacteria as a function of temperature.
 Notice that the curves are skewed toward the optimum temperature.

The skewing of the growth curve is thought to reflect the rapid denaturation of proteins as the temperature rises past the optimum for growth of the microorganism.

Life in extreme environments raises fascinating questions about the adaptation of macromolecules and metabolic processes. Very low temperatures affect cells in many ways. Membranes lose their fluidity and are damaged by ice crystal formation. Chemical reactions and diffusion slow considerably. Proteins become too rigid to catalyze reactions and may undergo denaturation. At the opposite end of the temperature spectrum, heat denatures proteins and nucleic acids. Increased fluidity impairs metabolic processes in membranes. Some of the practical applications of the destructive effects of heat on microbes are sterilization by steam, pasteurization, and incineration of inoculating loops. Proteins in

psychrophiles are, in general, rich in hydrophobic residues, display an increase in flexibility, and have a lower number of secondary stabilizing bonds when compared with homologous proteins from mesophiles.

Antifreeze proteins and solutes that decrease the freezing temperature of the cytoplasm are common. The lipids in the membranes tend to be unsaturated to increase fluidity. Growth rates are much slower than those encountered at moderate temperatures. Under appropriate conditions, mesophiles and even thermophiles can survive freezing. Liquid cultures of bacteria are mixed with sterile glycerol solutions and frozen to -80°C for long-term storage as stocks. Cultures can withstand freeze drying (lyophilization) and then be stored as powders in sealed ampules to be reconstituted with broth when needed.

Macromolecules in thermophiles and hyperthermophiles show some notable structural differences from what is observed in the mesophiles. The ratio of saturated to polyunsaturated lipids increases to limit the fluidity of the cell membranes. Their DNA sequences show a higher proportion of guanine–cytosine nitrogenous bases, which are held together by three hydrogen bonds in contrast to adenine and thymine, which are connected in the double helix by two hydrogen bonds. Additional secondary ionic and covalent bonds, as well as the replacement of key amino acids to stabilize folding, contribute to the resistance of proteins to denaturation. The so-called thermoenzymes purified from thermophiles have important practical applications. For example, amplification of nucleic acids in the polymerase chain reaction (PCR) depends on the thermal stability of *Taq* polymerase, an enzyme isolated from *T. aquaticus*. Degradation enzymes from thermophiles are added as ingredients in hot-water detergents, increasing their effectiveness.

Note:

- What temperature requirements do most bacterial human pathogens have?
- What DNA adaptation do thermophiles exhibit?

Note:**Feeding the World...and the World's Algae**

Artificial fertilizers have become an important tool in food production around the world. They are responsible for many of the gains of the so-called green revolution of the 20th century, which has allowed the planet to feed many of its more than 7 billion people. Artificial fertilizers provide nitrogen and phosphorus, key limiting nutrients, to crop plants, removing the normal barriers that would otherwise limit the rate of growth. Thus, fertilized crops grow much faster, and farms that use fertilizer produce higher crop yields.

However, careless use and overuse of artificial fertilizers have been demonstrated to have significant negative impacts on aquatic ecosystems, both freshwater and marine. Fertilizers that are applied at inappropriate times or in too-large quantities allow nitrogen and phosphorus compounds to escape use by crop plants and enter drainage systems. Inappropriate use of fertilizers in residential settings can also contribute to nutrient loads, which find their way to lakes and coastal marine ecosystems. As water warms and nutrients are plentiful, microscopic algae bloom, often changing the color of the water because of the high cell density.

Most algal blooms are not directly harmful to humans or wildlife; however, they can cause harm indirectly. As the algal population expands and then dies, it provides a large increase in organic matter to the bacteria that live in deep water. With this large supply of nutrients, the population of nonphotosynthetic microorganisms explodes, consuming available oxygen and creating “dead zones” where animal life has virtually disappeared.

Depletion of oxygen in the water is not the only damaging consequence of some algal blooms. The algae that produce red tides in the Gulf of Mexico, *Karenia brevis*, secrete potent toxins that can kill fish and other organisms and also accumulate in shellfish. Consumption of contaminated shellfish can cause severe neurological and gastrointestinal symptoms in humans. Shellfish beds must be regularly monitored for the presence of the toxins, and harvests are often shut down when it is present, incurring economic costs to the fishery. Cyanobacteria, which can form blooms in marine and freshwater ecosystems, produce toxins called microcystins, which can cause allergic reactions and liver damage when ingested in drinking water or during swimming. Recurring cyanobacterial algal blooms in Lake Erie

([link]) have forced municipalities to issue drinking water bans for days at a time because of unacceptable toxin levels.

This is just a small sampling of the negative consequences of algal blooms, red tides, and dead zones. Yet the benefits of crop fertilizer—the main cause of such blooms—are difficult to dispute. There is no easy solution to this dilemma, as a ban on fertilizers is not politically or economically feasible. In lieu of this, we must advocate for responsible use and regulation in agricultural and residential contexts, as well as the restoration of wetlands, which can absorb excess fertilizers before they reach lakes and oceans.



Heavy rains cause runoff of fertilizers into Lake Erie, triggering extensive algal blooms, which can be observed along the shoreline. Notice the brown unplanted and green planted agricultural land on the shore. (credit: NASA)

Note:



This [video](#) discusses algal blooms and dead zones in more depth.

Key Concepts and Summary

- Microorganisms thrive at a wide range of temperatures; they have colonized different natural environments and have adapted to extreme temperatures. Both extreme cold and hot temperatures require evolutionary adjustments to macromolecules and biological processes.
- **Psychrophiles** grow best in the temperature range of 0–15 °C whereas **psychrotrophs** thrive between 4°C and 25 °C.
- **Mesophiles** grow best at moderate temperatures in the range of 20 °C to about 45 °C. Pathogens are usually mesophiles.
- **Thermophiles** and **hyperthermophiles** are adapted to life at temperatures above 50 °C.
- Adaptations to cold and hot temperatures require changes in the composition of membrane lipids and proteins.

Critical Thinking

Exercise:

Problem:

The bacterium that causes Hansen's disease (leprosy), *Mycobacterium leprae*, infects mostly the extremities of the body: hands, feet, and nose. Can you make an educated guess as to its optimum temperature of growth?

Exercise:**Problem:**

Refer to [\[link\]](#). Some hyperthermophiles can survive autoclaving temperatures. Are they a concern in health care?

Other Environmental Conditions that Affect Growth

LEARNING OBJECTIVES

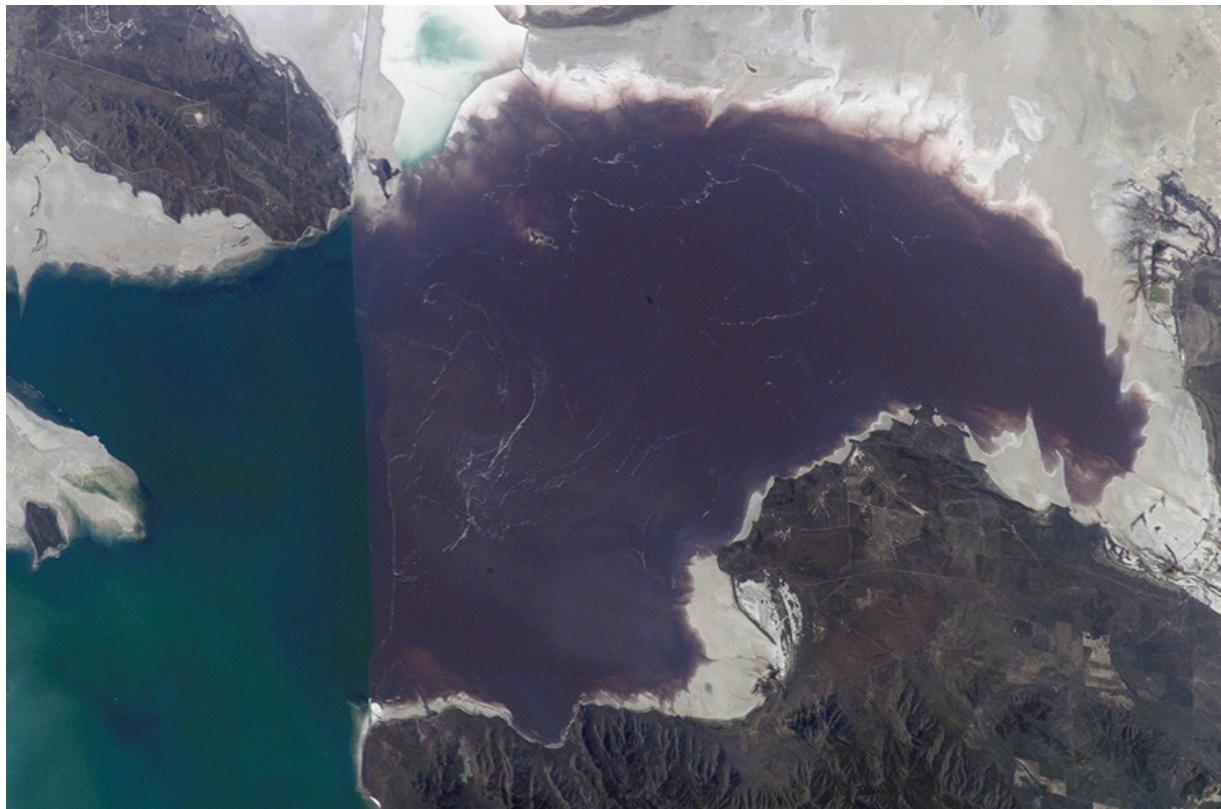
- Identify and describe different categories of microbes with specific growth requirements other than oxygen, pH, and temperature, such as altered barometric pressure, osmotic pressure, humidity, and light

Microorganisms interact with their environment along more dimensions than pH, temperature, and free oxygen levels, although these factors require significant adaptations. We also find microorganisms adapted to varying levels of salinity, barometric pressure, humidity, and light.

Osmotic and Barometric Pressure

Most natural environments tend to have lower solute concentrations than the cytoplasm of most microorganisms. Rigid cell walls protect the cells from bursting in a dilute environment. Not much protection is available against high osmotic pressure. In this case, water, following its concentration gradient, flows out of the cell. This results in plasmolysis (the shrinking of the protoplasm away from the intact cell wall) and cell death. This fact explains why brines and layering meat and fish in salt are time-honored methods of preserving food. Microorganisms called **halophiles** (“salt loving”) actually require high salt concentrations for growth. These organisms are found in marine environments where salt concentrations hover at 3.5%. Extreme halophilic microorganisms, such as the red alga *Dunaliella salina* and the archaeal species *Halobacterium* in [\[link\]](#), grow in

hypersaline lakes such as the Great Salt Lake, which is 3.5–8 times saltier than the ocean, and the Dead Sea, which is 10 times saltier than the ocean.



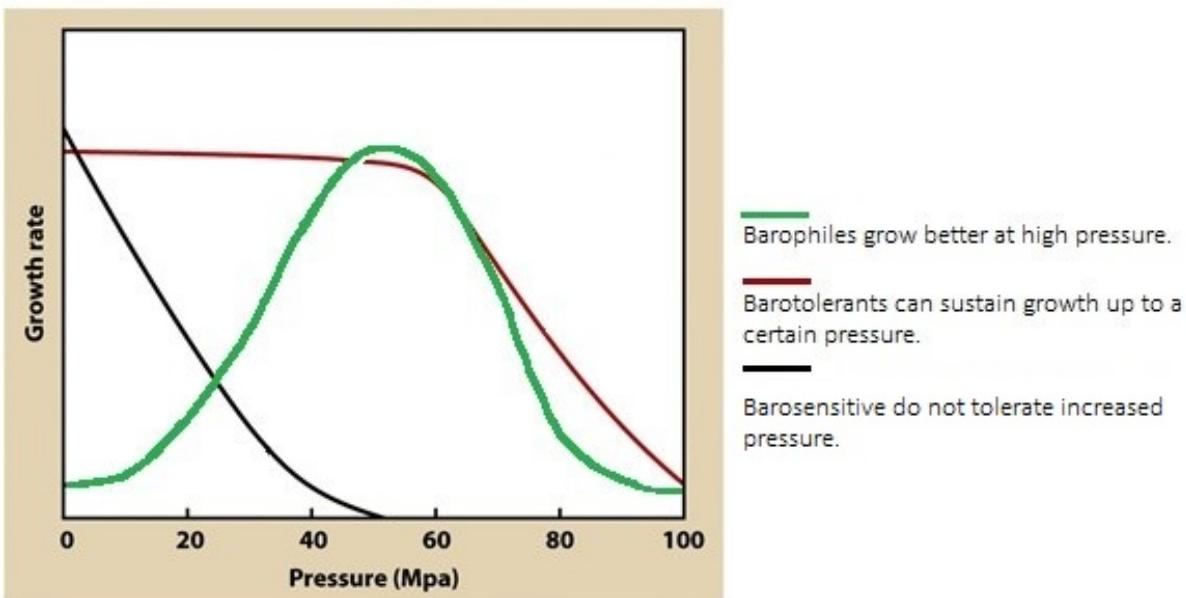
Photograph taken from space of the Great Salt Lake in Utah. The purple color is caused by high density of the alga *Dunaliella* and the archaean *Halobacterium* spp. (credit: NASA)

Dunaliella spp. counters the tremendous osmotic pressure of the environment with a high cytoplasmic concentration of glycerol and by actively pumping out salt ions. *Halobacterium* spp. accumulates large concentrations of K⁺ and other ions in its cytoplasm. Its proteins are designed for high salt concentrations and lose activity at salt concentrations below 1–2 M. Although most **halotolerant** organisms, for example *Halomonas* spp. in salt marshes, do not need high concentrations of salt for growth, they will survive and divide in the presence of high salt. Not

surprisingly, the staphylococci, micrococci, and corynebacteria that colonize our skin tolerate salt in their environment. Halotolerant pathogens are an important cause of food-borne illnesses because they survive and multiply in salty food. For example, the halotolerant bacteria *S. aureus*, *Bacillus cereus*, and *V. cholerae* produce dangerous enterotoxins and are major causes of food poisoning.

Microorganisms depend on available water to grow. Available moisture is measured as water activity (a_w), which is the ratio of the vapor pressure of the medium of interest to the vapor pressure of pure distilled water; therefore, the a_w of water is equal to 1.0. Bacteria require high a_w (0.97–0.99), whereas fungi can tolerate drier environments; for example, the range of a_w for growth of *Aspergillus* spp. is 0.8–0.75. Decreasing the water content of foods by drying, as in jerky, or through freeze-drying or by increasing osmotic pressure, as in brine and jams, are common methods of preventing spoilage.

Microorganisms that require high atmospheric pressure for growth are called **barophiles**. Many marine bacteria that are barophiles are also psychrophilic considering the average low temperature in deep ocean. Because it is difficult to retrieve intact specimens and reproduce such growth conditions in the laboratory, the characteristics of these microorganisms are largely unknown. It is known that cold temperatures and high hydrostatic pressure reduces plasma membrane fluidity, an important characteristic to any organism's survival. Bacteria that live in deep sea have high levels of polyunsaturated fatty acids that increase membrane fluidity. Other adaptations, such as keeping ribosomes intact, is also possible. Barosensitive bacteria did not evolve such adaptations, and barotolerant bacteria may have some of the adaptations.



Response of microbial growth at different pressures.

Light

Photoautotrophs, such as cyanobacteria or green sulfur bacteria, and photoheterotrophs, such as purple nonsulfur bacteria, depend on sufficient light intensity at the wavelengths absorbed by their pigments to grow and multiply. Energy from light is captured by pigments and converted into chemical energy that drives carbon fixation and other metabolic processes. The portion of the electromagnetic spectrum that is absorbed by these organisms is defined as photosynthetically active radiation (PAR). It lies within the visible light spectrum ranging from 400 to 700 nanometers (nm) and extends in the near infrared for some photosynthetic bacteria. A number of accessory pigments, such as fucoxanthin in brown algae and phycobilins in cyanobacteria, widen the useful range of wavelengths for photosynthesis and compensate for the low light levels available at greater depths of water. Other microorganisms, such as the archaea of the class Halobacteria, use light energy to drive their proton and sodium pumps. The light is absorbed by a pigment protein complex called bacteriorhodopsin, which is similar to the eye pigment rhodopsin. Photosynthetic bacteria are present not only in

aquatic environments but also in soil and in symbiosis with fungi in lichens. The peculiar watermelon snow is caused by a microalga *Chlamydomonas nivalis*, a green alga rich in a secondary red carotenoid pigment (astaxanthin) which gives the pink hue to the snow where the alga grows.

Note:

- Which photosynthetic pigments were described in this section?
- What is the fundamental stress of a hypersaline environment for a cell?

Key Concepts and Summary

- **Halophiles** require high salt concentration in the medium, whereas **halotolerant** organisms can grow and multiply in the presence of high salt but do not require it for growth.
- Halotolerant pathogens are an important source of foodborne illnesses because they contaminate foods preserved in salt.
- Photosynthetic bacteria depend on visible light for energy.
- Most bacteria, with few exceptions, require high moisture to grow.

Short Answer

Exercise:

Problem:

Fish sauce is a salty condiment produced using fermentation. What type of organism is likely responsible for the fermentation of the fish sauce?

Media Used for Bacterial Growth

LEARNING OBJECTIVES

- Identify and describe culture media for the growth of bacteria, including examples of all-purpose media, enriched, selective, differential, defined, and enrichment media

The study of microorganisms is greatly facilitated if we are able to culture them, that is, to keep reproducing populations alive under laboratory conditions. Culturing many microorganisms is challenging because of highly specific nutritional and environmental requirements and the diversity of these requirements among different species.

Nutritional Requirements

The number of available media to grow bacteria is considerable. Some media are considered general all-purpose media and support growth of a large variety of organisms. A prime example of an all-purpose medium is tryptic soy broth (TSB). Specialized media are used in the identification of bacteria and are supplemented with dyes, pH indicators, or antibiotics. One type, **enriched media**, contains growth factors, vitamins, and other essential nutrients to promote the growth of **fastidious organisms**, organisms that cannot make certain nutrients and require them to be added to the medium. When the complete chemical composition of a medium is known, it is called a **chemically defined medium**. For example, in EZ medium, all individual chemical components are identified and the exact amounts of each is known. In **complex media**, which contain extracts and digests of yeasts, meat, or plants, the precise chemical composition of the

medium is not known. Amounts of individual components are undetermined and variable. Nutrient broth, tryptic soy broth, and brain heart infusion, are all examples of complex media.

Media that inhibit the growth of unwanted microorganisms and support the growth of the organism of interest by supplying nutrients and reducing competition are called **selective media**. An example of a selective medium is MacConkey agar. It contains bile salts and crystal violet, which interfere with the growth of many gram-positive bacteria and favor the growth of gram-negative bacteria, particularly the Enterobacteriaceae. These species are commonly named enterics, reside in the intestine, and are adapted to the presence of bile salts. The **enrichment cultures** foster the preferential growth of a desired microorganism that represents a fraction of the organisms present in an inoculum. For example, if we want to isolate bacteria that break down crude oil, hydrocarbonoclastic bacteria, sequential subculturing in a medium that supplies carbon only in the form of crude oil will enrich the cultures with oil-eating bacteria. The **differential media** make it easy to distinguish colonies of different bacteria by a change in the color of the colonies or the color of the medium. Color changes are the result of end products created by interaction of bacterial enzymes with differential substrates in the medium or, in the case of hemolytic reactions, the lysis of red blood cells in the medium. In [[link](#)], the differential fermentation of lactose can be observed on MacConkey agar. The lactose fermenters produce acid, which turns the medium and the colonies of strong fermenters hot pink. The medium is supplemented with the pH indicator neutral red, which turns to hot pink at low pH. Selective and differential media can be combined and play an important role in the identification of bacteria by biochemical methods.



ASM MicrobeLibrary.org © Miller and Hanley

On this MacConkey agar plate, the lactose-fermenter *E. coli* colonies are bright pink. *Serratia marcescens*, which does not ferment lactose, forms a cream-colored streak on the tan medium. (credit: American Society for Microbiology)

Note:

- Distinguish complex and chemically defined media.
- Distinguish selective and enrichment media.

Note:

Compare the compositions of [EZ medium](#) and [sheep blood](#) agar.

Note:

The End-of-Year Picnic

The microbiology department is celebrating the end of the school year in May by holding its traditional picnic on the green. The speeches drag on for a couple of hours, but finally all the faculty and students can dig into the food: chicken salad, tomatoes, onions, salad, and custard pie. By evening, the whole department, except for two vegetarian students who did not eat the chicken salad, is stricken with nausea, vomiting, retching, and abdominal cramping. Several individuals complain of diarrhea. One patient shows signs of shock (low blood pressure). Blood and stool samples are collected from patients, and an analysis of all foods served at the meal is conducted.

Bacteria can cause gastroenteritis (inflammation of the stomach and intestinal tract) either by colonizing and replicating in the host, which is considered an infection, or by secreting toxins, which is considered intoxication. Signs and symptoms of infections are typically delayed, whereas intoxication manifests within hours, as happened after the picnic. Blood samples from the patients showed no signs of bacterial infection, which further suggests that this was a case of intoxication. Since intoxication is due to secreted toxins, bacteria are not usually detected in blood or stool samples. MacConkey agar and sorbitol-MacConkey agar plates and xylose-lysine-deoxycholate (XLD) plates were inoculated with stool samples and did not reveal any unusually colored colonies, and no

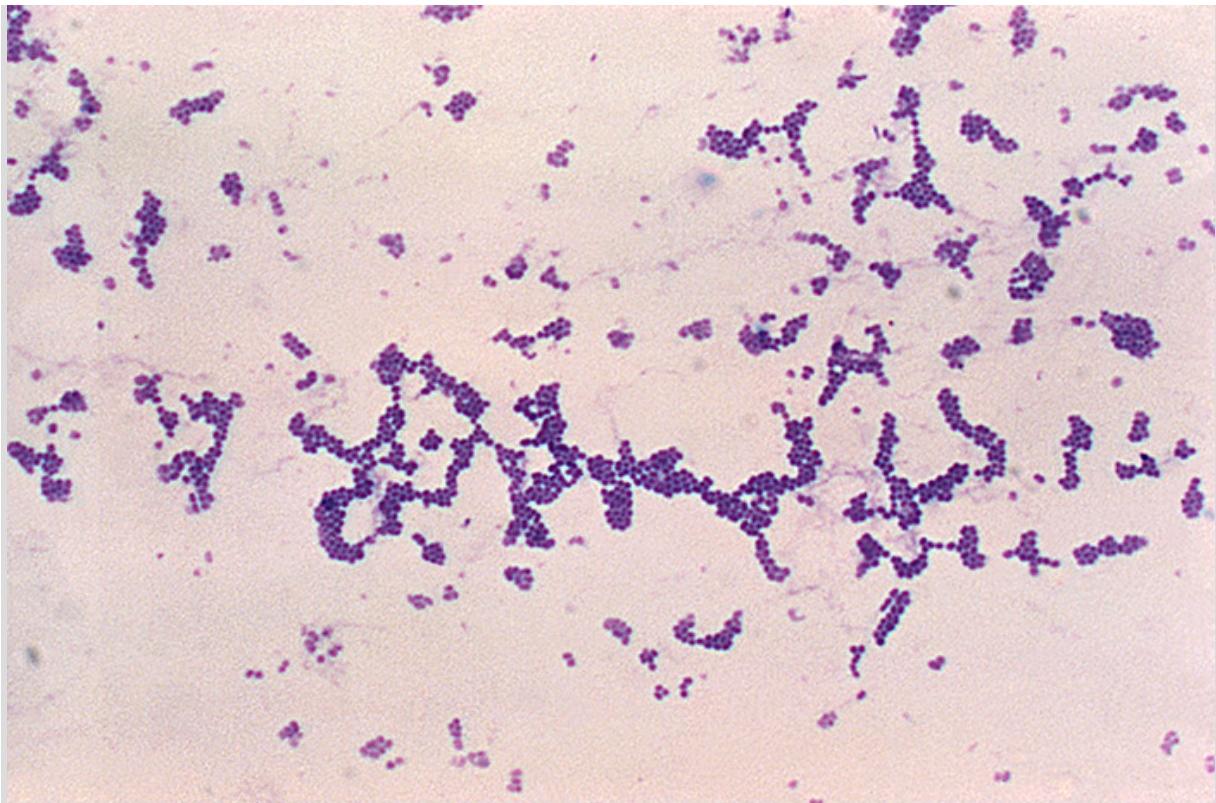
black colonies or white colonies were observed on XLD. All lactose fermenters on MacConkey agar also ferment sorbitol. These results ruled out common agents of food-borne illnesses: *E. coli*, *Salmonella* spp., and *Shigella* spp.

Analysis of the chicken salad revealed an abnormal number of gram-positive cocci arranged in clusters ([\[link\]](#)). A culture of the gram-positive cocci releases bubbles when mixed with hydrogen peroxide. The culture turned mannitol salt agar yellow after a 24-hour incubation.

All the tests point to *Staphylococcus aureus* as the organism that secreted the toxin. Samples from the salad showed the presence of gram-positive cocci bacteria in clusters. The colonies were positive for catalase. The bacteria grew on mannitol salt agar fermenting mannitol, as shown by the change to yellow of the medium. The pH indicator in mannitol salt agar is phenol red, which turns to yellow when the medium is acidified by the products of fermentation.

The toxin secreted by *S. aureus* is known to cause severe gastroenteritis. The organism was probably introduced into the salad during preparation by the food handler and multiplied while the salad was kept in the warm ambient temperature during the speeches.

- What are some other factors that might have contributed to rapid growth of *S. aureus* in the chicken salad?
- Why would *S. aureus* not be inhibited by the presence of salt in the chicken salad?



Gram-positive cocci in clusters. (credit: Centers for Disease Control and Prevention)

Key Concepts and Summary

- **Chemically defined media** contain only chemically known components.
- **Selective media** favor the growth of some microorganisms while inhibiting others.
- **Enriched media** contain added essential nutrients a specific organism needs to grow
- **Differential media** help distinguish bacteria by the color of the colonies or the change in the medium.

Multiple Choice

Exercise:

Problem:

EMB agar is a medium used in the identification and isolation of pathogenic bacteria. It contains digested meat proteins as a source of organic nutrients. Two indicator dyes, eosin and methylene blue, inhibit the growth of gram-positive bacteria and distinguish between lactose fermenting and nonlactose fermenting organisms. Lactose fermenters form metallic green or deep purple colonies, whereas the nonlactose fermenters form completely colorless colonies. EMB agar is an example of which of the following?

- A. a selective medium only
 - B. a differential medium only
 - C. a selective medium and a chemically defined medium
 - D. a selective medium, a differential medium, and a complex medium
-

Solution:

D

Exercise:

Problem:

Haemophilus influenzae must be grown on chocolate agar, which is blood agar treated with heat to release growth factors in the medium. *H. influenzae* is described as _____.

- A. an acidophile
- B. a thermophile
- C. an obligate anaerobe
- D. fastidious

Solution:

D

Fill in the Blank**Exercise:****Problem:**

Blood agar contains many unspecified nutrients, supports the growth of a large number of bacteria, and allows differentiation of bacteria according to hemolysis (breakdown of blood). The medium is _____ and _____.

Solution:

complex, differential

Exercise:**Problem:**

Rogosa agar contains yeast extract. The pH is adjusted to 5.2 and discourages the growth of many microorganisms; however, all the colonies look similar. The medium is _____ and _____.

Solution:

complex, selective

Short Answer**Exercise:**

Problem:

What is the major difference between an enrichment culture and a selective culture?

Critical Thinking

Exercise:**Problem:**

Haemophilus, influenzae grows best at 35–37 °C with ~5% CO₂ (or in a candle-jar) and requires hemin (X factor) and nicotinamide-adenine-dinucleotide (NAD, also known as V factor) for growth.[\[footnote\]](#)

Using the vocabulary learned in this chapter, describe *H. influenzae*.

Centers for Disease Control and Prevention, World Health

Organization. “*CDC Laboratory Methods for the Diagnosis of*

Meningitis Caused by Neisseria meningitidis, Streptococcus

pneumoniae, and Haemophilus influenzae. WHO Manual, 2nd edition.”

2011. <http://www.cdc.gov/meningitis/lab-manual/full-manual.pdf>

Biochemistry of the genome - Introduction

class="introduction"

Siblings
within a
family share
some genes
with each
other and with
each parent.

Identical
twins,
however, are
genetically
identical.

Bacteria like
Escherichia
coli may
acquire genes
encoding
virulence
factors,
converting
them into
pathogenic
strains, like
this

uropathogenic
E. coli. (credit

left:
modification
of work by
Pellegrini C,
Fargnoli MC,
Suppa M,
Peris K; credit
right:

modification
of work by
American
Society for
Microbiology
)



Children inherit some characteristics from each parent. Siblings typically look similar to each other, but not exactly the same—except in the case of identical twins. How can we explain these phenomena? The answers lie in heredity (the transmission of traits from one generation to the next) and genetics (the science of heredity). Because humans reproduce sexually, 50% of a child's genes come from the mother's egg cell and the remaining 50% from the father's sperm cell. Sperm and egg are formed through the process of meiosis, where DNA recombination occurs. Thus, there is no predictable pattern as to which 50% comes from which parent. Thus, siblings have only some genes, and their associated characteristics, in common. Identical twins are the exception, because they are genetically identical.

Genetic differences among related microbes also dictate many observed biochemical and virulence differences. For example, some strains of the bacterium *Escherichia coli* are harmless members of the normal microbiota in the human gastrointestinal tract. Other strains of the same species have genes that give them the ability to cause disease. In bacteria, such genes are not inherited via sexual reproduction, as in humans. Often, they are

transferred via plasmids, small circular pieces of double-stranded DNA that can be exchanged between prokaryotes.

Using Microbiology to Discover the Secrets of Life

LEARNING OBJECTIVES

- Describe the discovery of nucleic acid and nucleotides
- Explain the historical experiments that led to the characterization of DNA
- Describe how microbiology and microorganisms have been used to discover the biochemistry of genes
- Explain how scientists established the link between DNA and heredity

Note:

Part 1

Alex is a 22-year-old college student who vacationed in Puerto Vallarta, Mexico, for spring break. Unfortunately, two days after flying home to Ohio, he began to experience abdominal cramping and extensive watery diarrhea. Because of his discomfort, he sought medical attention at a large Cincinnati hospital nearby.

- What types of infections or other conditions may be responsible?

Jump to the [next](#) Clinical Focus box.

Through the early 20th century, DNA was not yet recognized as the genetic material responsible for heredity, the passage of traits from one generation to the next. In fact, much of the research was dismissed until the mid-20th

century. The scientific community believed, incorrectly, that the process of inheritance involved a blending of parental traits that produced an intermediate physical appearance in offspring; this hypothetical process appeared to be correct because of what we know now as continuous variation, which results from the action of many genes to determine a particular characteristic, like human height. Offspring appear to be a “blend” of their parents’ traits when we look at characteristics that exhibit continuous variation. The blending theory of inheritance asserted that the original parental traits were lost or absorbed by the blending in the offspring, but we now know that this is not the case.

Two separate lines of research, begun in the mid to late 1800s, ultimately led to the discovery and characterization of DNA and the foundations of genetics, the science of heredity. These lines of research began to converge in the 1920s, and research using microbial systems ultimately resulted in significant contributions to elucidating the molecular basis of genetics.

Discovery and Characterization of DNA

Modern understanding of DNA has evolved from the discovery of nucleic acid to the development of the double-helix model. In the 1860s, Friedrich Miescher (1844–1895), a physician by profession, was the first person to isolate phosphorus-rich chemicals from leukocytes (white blood cells) from the pus on used bandages from a local surgical clinic. He named these chemicals (which would eventually be known as RNA and DNA) “nuclein” because they were isolated from the nuclei of the cells. His student Richard Altmann (1852–1900) subsequently termed it “nucleic acid” 20 years later when he discovered the acidic nature of nuclein. In the last two decades of the 19th century, German biochemist Albrecht Kossel (1853–1927) isolated and characterized the five different nucleotide bases composing nucleic acid. These are adenine, guanine, cytosine, thymine (in DNA), and uracil (in RNA). Kossel received the Nobel Prize in Physiology or Medicine in 1910 for his work on nucleic acids and for his considerable work on proteins, including the discovery of histidine.

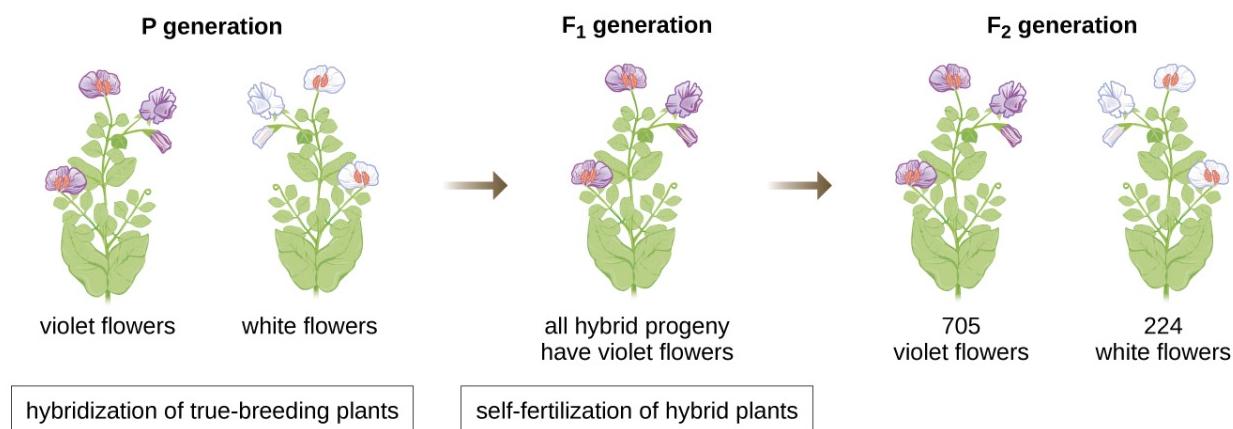
Foundations of Genetics

Despite the discovery of DNA in the late 1800s, scientists did not make the association with heredity for many more decades. To make this connection, scientists, including a number of microbiologists, performed many experiments on plants, animals, and bacteria.

Mendel's Pea Plants

While Miescher was isolating and discovering DNA in the 1860s, Austrian monk and botanist Johann Gregor Mendel (1822–1884) was experimenting with garden peas, demonstrating and documenting basic patterns of inheritance, now known as Mendel's laws.

In 1856, Mendel began his decade-long research into inheritance patterns. He used the diploid garden pea, *Pisum sativum*, as his primary model system because it naturally self-fertilizes and is highly inbred, producing “true-breeding” pea plant lines—plants that always produce offspring that look like the parent. By experimenting with true-breeding pea plants, Mendel avoided the appearance of unexpected traits in offspring that might occur if he used plants that were not true-breeding. Mendel performed hybridizations, which involve mating two true-breeding individuals (P generation) that have different traits, and examined the characteristics of their offspring (first filial generation, F₁) as well as the offspring of self-fertilization of the F₁ generation (second filial generation, F₂) ([\[link\]](#)).



In one of his experiments on inheritance patterns, Mendel crossed plants that were true-breeding for violet flower color with plants true-breeding for white flower color (the P generation). The resulting hybrids in the F₁ generation all had violet flowers. In the F₂ generation, approximately three-quarters of the plants had violet flowers, and one-quarter had white flowers.

In 1865, Mendel presented the results of his experiments with nearly 30,000 pea plants to the local natural history society. He demonstrated that traits are transmitted faithfully from parents to offspring independently of other traits. In 1866, he published his work, “Experiments in Plant Hybridization,”^[footnote] in the *Proceedings of the Natural History Society of Brünn*. Mendel’s work went virtually unnoticed by the scientific community, which believed, incorrectly, in the theory of blending of traits in continuous variation.

J.G. Mendel. “Versuche über Pflanzenhybriden.” *Verhandlungen des naturforschenden Vereines in Brünn, Bd. Abhandlungen 4* (1865):3–7. (For English translation, see <http://www.mendelweb.org/Mendel.plain.html>)

He was not recognized for his extraordinary scientific contributions during his lifetime. In fact, it was not until 1900 that his work was rediscovered, reproduced, and revitalized by scientists on the brink of discovering the chromosomal basis of heredity.

The Chromosomal Theory of Inheritance

Mendel carried out his experiments long before chromosomes were visualized under a microscope. However, with the improvement of microscopic techniques during the late 1800s, cell biologists could stain and visualize subcellular structures with dyes and observe their actions during meiosis. They were able to observe chromosomes replicating, condensing from an amorphous nuclear mass into distinct X-shaped bodies and migrating to separate cellular poles. The speculation that chromosomes might be the key to understanding heredity led several scientists to examine

Mendel's publications and re-evaluate his model in terms of the behavior of chromosomes during mitosis and meiosis.

In 1902, Theodor Boveri (1862–1915) observed that in sea urchins, nuclear components (chromosomes) determined proper embryonic development. That same year, Walter Sutton (1877–1916) observed the separation of chromosomes into daughter cells during meiosis. Together, these observations led to the development of the Chromosomal Theory of Inheritance, which identified chromosomes as the genetic material responsible for Mendelian inheritance.

Despite compelling correlations between the behavior of chromosomes during meiosis and Mendel's observations, the Chromosomal Theory of Inheritance was proposed long before there was any direct evidence that traits were carried on chromosomes. Thomas Hunt Morgan (1866–1945) and his colleagues spent several years carrying out crosses with the fruit fly, *Drosophila melanogaster*. They performed meticulous microscopic observations of fly chromosomes and correlated these observations with resulting fly characteristics. Their work provided the first experimental evidence to support the Chromosomal Theory of Inheritance in the early 1900s. In 1915, Morgan and his "Fly Room" colleagues published *The Mechanism of Mendelian Heredity*, which identified chromosomes as the cellular structures responsible for heredity. For his many significant contributions to genetics, Morgan received the Nobel Prize in Physiology or Medicine in 1933.

In the late 1920s, Barbara McClintock (1902–1992) developed chromosomal staining techniques to visualize and differentiate between the different chromosomes of maize (corn). In the 1940s and 1950s, she identified a breakage event on chromosome 9, which she named the dissociation locus (*Ds*). *Ds* could change position within the chromosome. She also identified an activator locus (*Ac*). *Ds* chromosome breakage could be activated by an *Ac* element (transposase enzyme). At first, McClintock's finding of these jumping genes, which we now call transposons, was not accepted by the scientific community. It wasn't until the 1960s and later that transposons were discovered in bacteriophages, bacteria, and *Drosophila*. Today, we know that transposons are mobile segments of DNA

that can move within the genome of an organism. They can regulate gene expression, protein expression, and virulence (ability to cause disease).

Microbes and Viruses in Genetic Research

Microbiologists have also played a crucial part in our understanding of genetics. Experimental organisms such as Mendel’s garden peas, Morgan’s fruit flies, and McClintock’s corn had already been used successfully to pave the way for an understanding of genetics. However, microbes and viruses were (and still are) excellent model systems for the study of genetics because, unlike peas, fruit flies, and corn, they are propagated more easily in the laboratory, growing to high population densities in a small amount of space and in a short time. In addition, because of their structural simplicity, microbes and viruses are more readily manipulated genetically.

Fortunately, despite significant differences in size, structure, reproduction strategies, and other biological characteristics, there is biochemical unity among all organisms; they have in common the same underlying molecules responsible for heredity and the use of genetic material to give cells their varying characteristics. In the words of French scientist Jacques Monod, “What is true for *E. coli* is also true for the elephant,” meaning that the biochemistry of life has been maintained throughout evolution and is shared in all forms of life, from simple unicellular organisms to large, complex organisms. This biochemical continuity makes microbes excellent models to use for genetic studies.

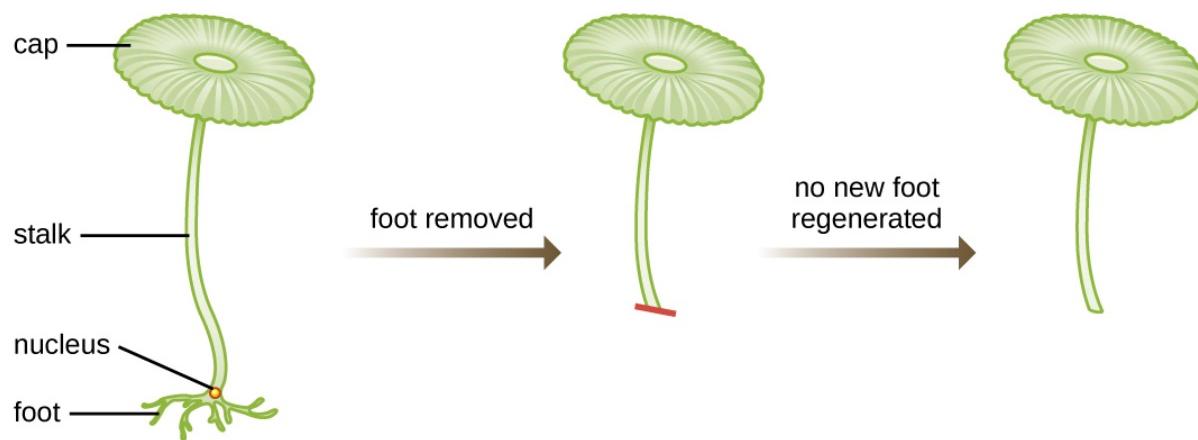
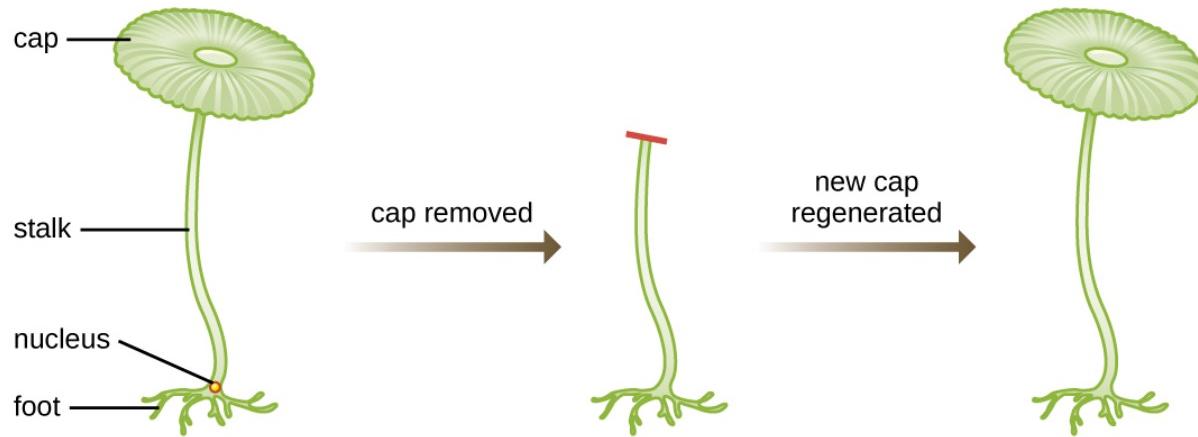
In a clever set of experiments in the 1930s and 1940s, German scientist Joachim Hämmerling (1901–1980), using the single-celled alga *Acetabularia* as a microbial model, established that the genetic information in a eukaryotic cell is housed within the nucleus. *Acetabularia* spp. are unusually large algal cells that grow asymmetrically, forming a “foot” containing the nucleus, which is used for substrate attachment; a stalk; and an umbrella-like cap—structures that can all be easily seen with the naked eye. In an early set of experiments, Hämmerling removed either the cap or the foot of the cells and observed whether new caps or feet were

regenerated ([\[link\]](#)). He found that when the foot of these cells was removed, new feet did not grow; however, when caps were removed from the cells, new caps were regenerated. This suggested that the hereditary information was located in the nucleus-containing foot of each cell.



Acetabularia

(a)



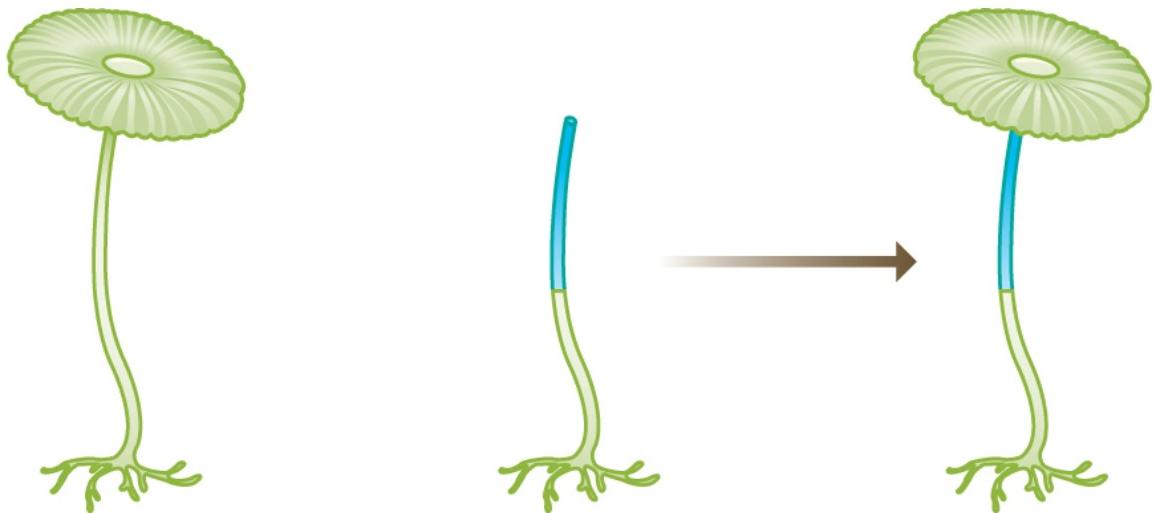
(b)

(a) The cells of the single-celled alga *Acetabularia* measure 2–6 cm

and have a cell morphology that can be observed with the naked eye. Each cell has a cap, a stalk, and a foot, which contains the nucleus. (b)

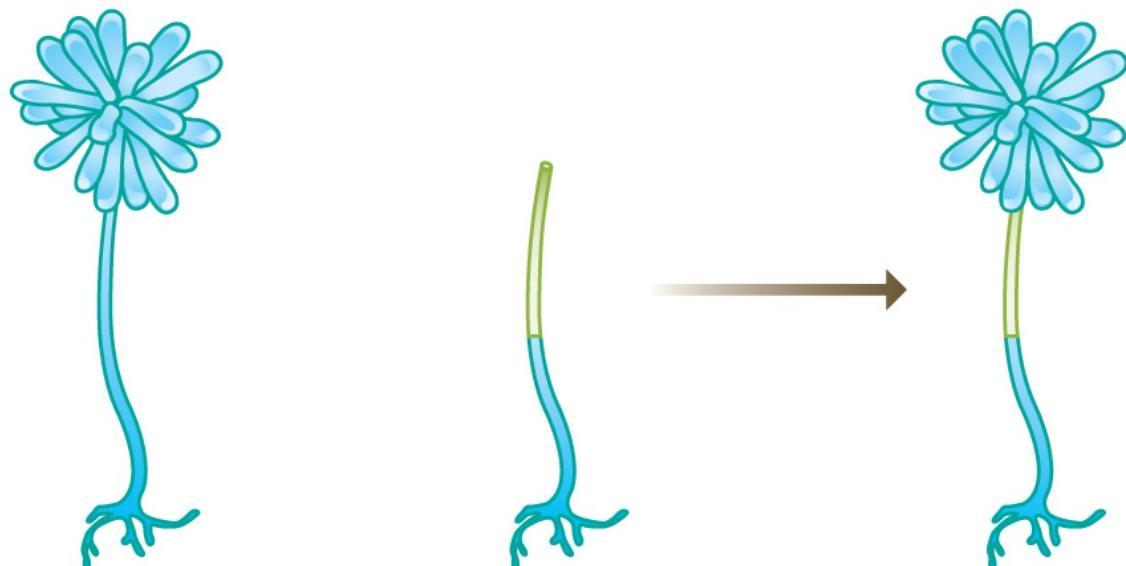
Hämmerling found that if he removed the cap, a new cap would regenerate; but if he removed the foot, a new foot would not regenerate. He concluded that the genetic information needed for regeneration was found in the nucleus. (credit a: modification of work by James St. John)

In another set of experiments, Hämmerling used two species of *Acetabularia* that have different cap morphologies, *A. crenulata* and *A. mediterranea* ([\[link\]](#)). He cut the caps from both types of cells and then grafted the stalk from an *A. crenulata* onto an *A. mediterranea* foot, and vice versa. Over time, he observed that the grafted cell with the *A. crenulata* foot and *A. mediterranea* stalk developed a cap with the *A. crenulata* morphology. Conversely, the grafted cell with the *A. mediterranea* foot and *A. crenulata* stalk developed a cap with the *A. mediterranea* morphology. He microscopically confirmed the presence of nuclei in the feet of these cells and attributed the development of these cap morphologies to the nucleus of each grafted cell. Thus, he showed experimentally that the nucleus was the location of genetic material that dictated a cell's properties.



Acetabularia mediterranea

graft



Acetabularia crenulata

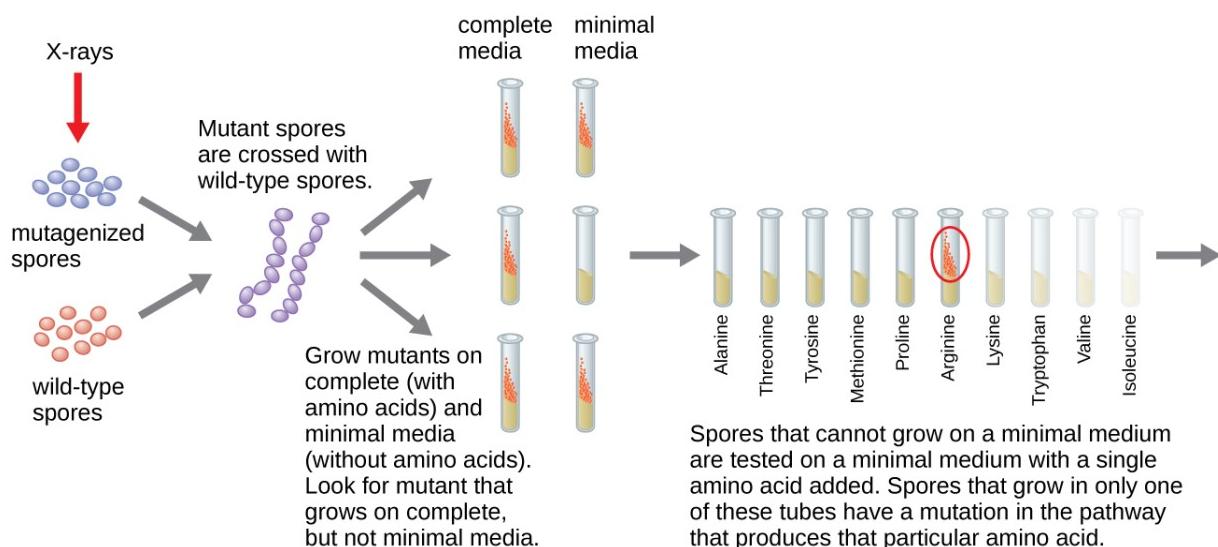
graft

In a second set of experiments, Hä默ling used two morphologically different species and grafted stalks from each species to the feet of the other. He found that the properties of the regenerated caps were dictated by the species of the nucleus-containing foot.

Another microbial model, the red bread mold *Neurospora crassa*, was used by George Beadle and Edward Tatum to demonstrate the relationship between genes and the proteins they encode. Beadle had worked with fruit flies in Morgan's laboratory but found them too complex to perform certain types of experiments. *N. crassa*, on the other hand, is a simpler organism and has the ability to grow on a minimal medium because it contains enzymatic pathways that allow it to use the medium to produce its own vitamins and amino acids.

Beadle and Tatum irradiated the mold with X-rays to induce changes to a sequence of nucleic acids, called mutations. They mated the irradiated mold spores and attempted to grow them on both a complete medium and a minimal medium. They looked for mutants that grew on a complete medium, supplemented with vitamins and amino acids, but did not grow on the minimal medium lacking these supplements. Such molds theoretically contained mutations in the genes that encoded biosynthetic pathways. Upon finding such mutants, they systematically tested each to determine which vitamin or amino acid it was unable to produce ([\[link\]](#)) and published this work in 1941. [\[footnote\]](#)

G.W. Beadle, E.L. Tatum. "Genetic Control of Biochemical Reactions in *Neurospora*." *Proceedings of the National Academy of Sciences* 27 no. 11 (1941):499–506.

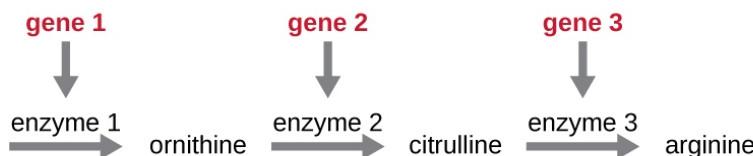


Beadle and Tatum's experiment involved the mating of irradiated and nonirradiated mold spores. These spores were grown on both complete medium and a minimal medium to determine which amino acid or vitamin the mutant was unable to produce on its own.

Subsequent work by Beadle, Tatum, and colleagues showed that they could isolate different classes of mutants that required a particular supplement, like the amino acid arginine ([\[link\]](#)). With some knowledge of the arginine biosynthesis pathway, they identified three classes of arginine mutants by supplementing the minimal medium with intermediates (citrulline or ornithine) in the pathway. The three mutants differed in their abilities to grow in each of the media, which led the group of scientists to propose, in 1945, that each type of mutant had a defect in a different gene in the arginine biosynthesis pathway. This led to the so-called one gene–one enzyme hypothesis, which suggested that each gene encodes one enzyme.

Subsequent knowledge about the processes of transcription and translation led scientists to revise this to the “one gene–one polypeptide” hypothesis. Although there are some genes that do not encode polypeptides (but rather encode for transfer RNAs [tRNAs] or ribosomal RNAs [rRNAs], which we will discuss later), the one gene–one enzyme hypothesis is true in many cases, especially in microbes. Beadle and Tatum’s discovery of the link between genes and corresponding characteristics earned them the 1958 Nobel Prize in Physiology and Medicine and has since become the basis for modern molecular genetics.

Beadle and Tatum Experiments				
Bread Mold	Minimal Medium (MM)	MM + Ornithine	MM + Citrulline	MM + Arginine
Wild type	grew	grew	grew	grew
Mutant 1	did not grow	grew	grew	grew
Mutant 2	did not grow	did not grow	grew	grew
Mutant 3	did not grow	did not grow	did not grow	grew



Three classes of arginine mutants were identified, each differing in their ability to grow in the presence of intermediates in the arginine biosynthesis pathway. From this, Beadle and Tatum concluded that each mutant was defective in a different gene encoding a different enzyme in the arginine biosynthesis pathway, leading to their one gene—one enzyme hypothesis.

Note:



To learn more about the experiments of Beadle and Tatum, visit this [website](#) from the DNA Learning Center.

Note:

- What organism did Morgan and his colleagues use to develop the Chromosomal Theory of Inheritance? What traits did they track?
- What did Hämmerling prove with his experiments on *Acetabularia*?

DNA as the Molecule Responsible for Heredity

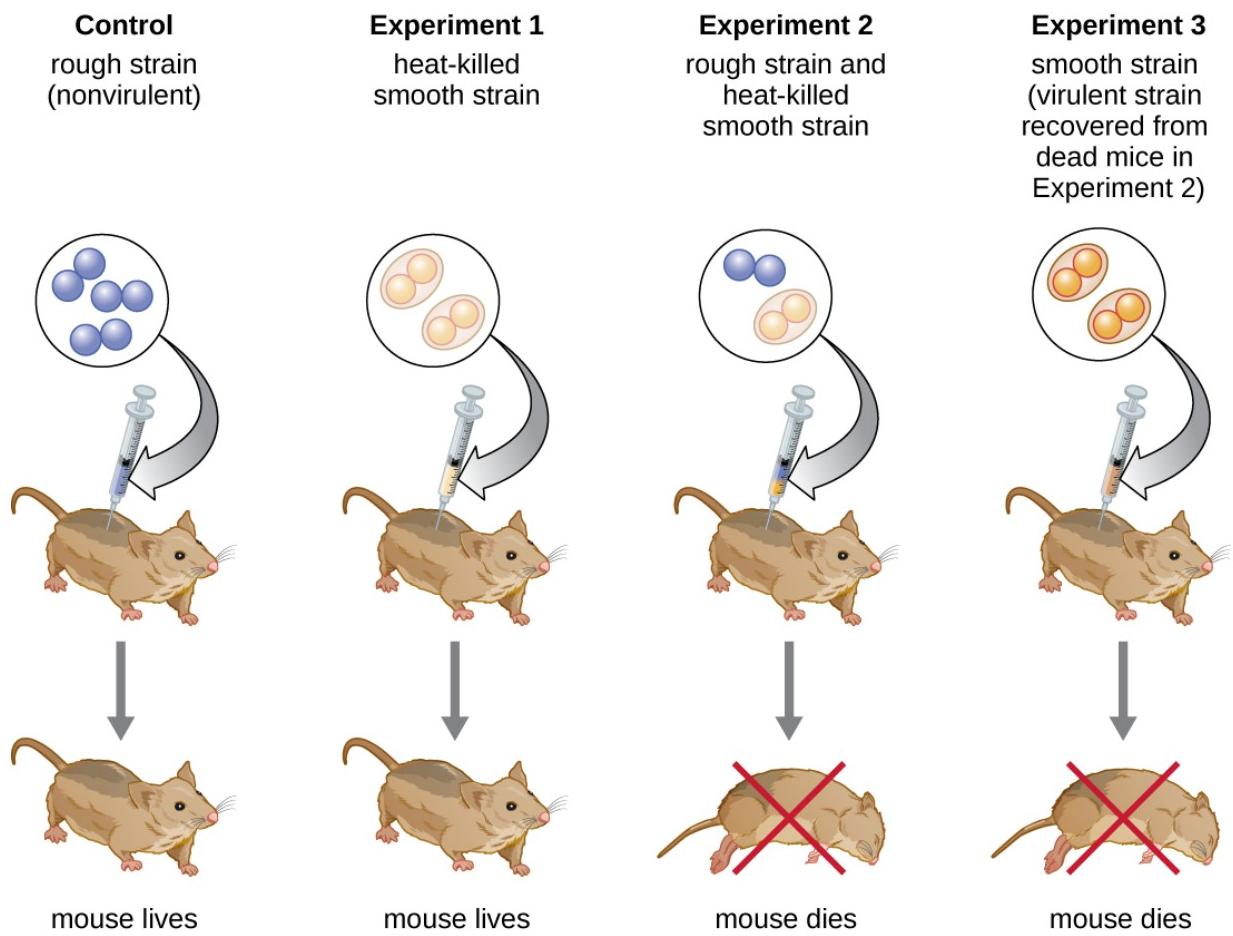
By the beginning of the 20th century, a great deal of work had already been done on characterizing DNA and establishing the foundations of genetics, including attributing heredity to chromosomes found within the nucleus. Despite all of this research, it was not until well into the 20th century that these lines of research converged and scientists began to consider that DNA could be the genetic material that offspring inherited from their parents. DNA, containing only four different nucleotides, was thought to be structurally too simple to encode such complex genetic information. Instead, protein was thought to have the complexity required to serve as cellular genetic information because it is composed of 20 different amino acids that could be combined in a huge variety of combinations. Microbiologists played a pivotal role in the research that determined that DNA is the molecule responsible for heredity.

Griffith's Transformation Experiments

British bacteriologist Frederick Griffith (1879–1941) was perhaps the first person to show that hereditary information could be transferred from one cell to another “horizontally” (between members of the same generation), rather than “vertically” (from parent to offspring). In 1928, he reported the first demonstration of bacterial transformation, a process in which external DNA is taken up by a cell, thereby changing its characteristics.[\[footnote\]](#) He was working with two strains of *Streptococcus pneumoniae*, a bacterium that causes pneumonia: a rough (R) strain and a smooth (S) strain. The R strain is nonpathogenic and lacks a capsule on its outer surface; as a result, colonies from the R strain appear rough when grown on plates. The S strain is pathogenic and has a capsule outside its cell wall, allowing it to escape phagocytosis by the host immune system. The capsules cause colonies from the S strain to appear smooth when grown on plates.

F. Griffith. “The Significance of Pneumococcal Types.” *Journal of Hygiene* 27 no. 2 (1928):8–159.

In a series of experiments, Griffith analyzed the effects of live R, live S, and heat-killed S strains of *S. pneumoniae* on live mice ([\[link\]](#)). When mice were injected with the live S strain, the mice died. When he injected the mice with the live R strain or the heat-killed S strain, the mice survived. But when he injected the mice with a mixture of live R strain and heat-killed S strain, the mice died. Upon isolating the live bacteria from the dead mouse, he only recovered the S strain of bacteria. When he then injected this isolated S strain into fresh mice, the mice died. Griffith concluded that something had passed from the heat-killed S strain into the live R strain and “transformed” it into the pathogenic S strain; he called this the “transforming principle.” These experiments are now famously known as Griffith’s transformation experiments.

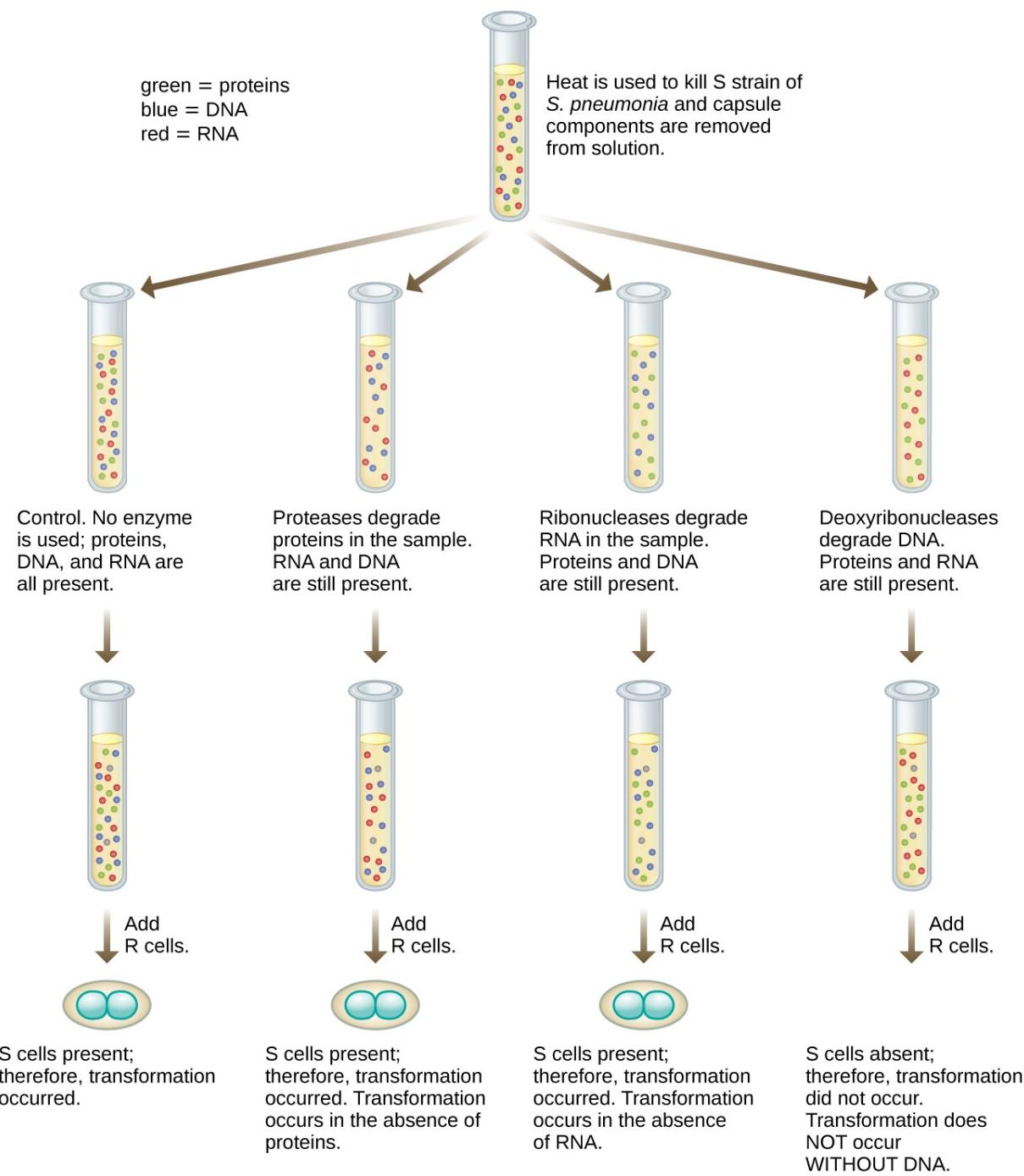


In his famous series of experiments, Griffith used two strains of *S. pneumoniae*. The S strain is pathogenic and causes death. Mice injected with the nonpathogenic R strain or the heat-killed S strain survive. However, a combination of the heat-killed S strain and the live R strain causes the mice to die. The S strain recovered from the dead mouse showed that something had passed from the heat-killed S strain to the R strain, transforming the R strain into an S strain in the process.

In 1944, Oswald Avery, Colin MacLeod, and Maclyn McCarty were interested in exploring Griffith's transforming principle further. They isolated the S strain from infected dead mice, heat-killed it, and inactivated various components of the S extract, conducting a systematic elimination study ([\[link\]](#)). They used enzymes that specifically degraded proteins,

RNA, and DNA and mixed the S extract with each of these individual enzymes. Then, they tested each extract/enzyme combination's resulting ability to transform the R strain, as observed by the diffuse growth of the S strain in culture media and confirmed visually by growth on plates. They found that when DNA was degraded, the resulting mixture was no longer able to transform the R strain bacteria, whereas no other enzymatic treatment was able to prevent transformation. This led them to conclude that DNA was the transforming principle. Despite their results, many scientists did not accept their conclusion, instead believing that there were protein contaminants within their extracts.

Determining the identity of the hereditary material



Oswald Avery, Colin MacLeod, and Maclyn McCarty followed up on Griffith's experiment and experimentally determined that the transforming principle was DNA.

Note:

- How did Avery, MacLeod, and McCarty's experiments show that DNA was the transforming principle first described by Griffith?

Hershey and Chase's Proof of DNA as Genetic Material

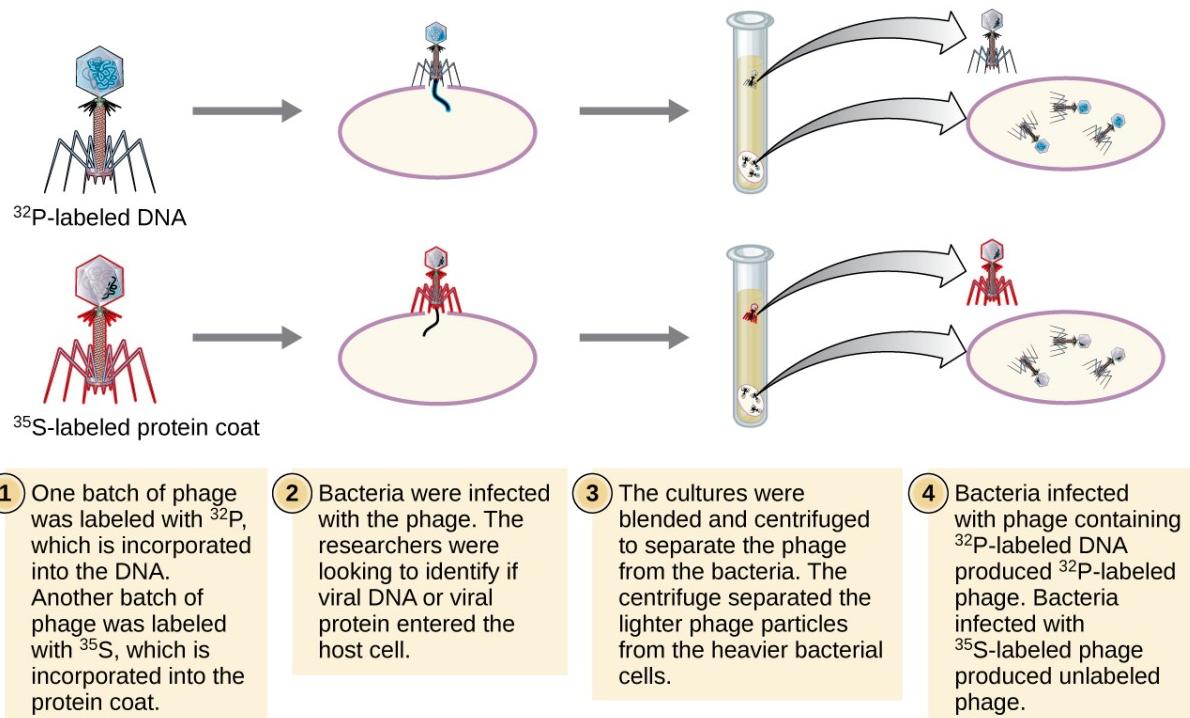
Alfred Hershey and Martha Chase performed their own experiments in 1952 and were able to provide confirmatory evidence that DNA, not protein, was the genetic material ([\[link\]](#)).[\[footnote\]](#) Hershey and Chase were studying a bacteriophage, a virus that infects bacteria. Viruses typically have a simple structure: a protein coat, called the capsid, and a nucleic acid core that contains the genetic material, either DNA or RNA (see [Viruses](#)). The particular bacteriophage they were studying was the T2 bacteriophage, which infects *E. coli* cells. As we now know today, T2 attaches to the surface of the bacterial cell and then it injects its nucleic acids inside the cell. The phage DNA makes multiple copies of itself using the host machinery, and eventually the host cell bursts, releasing a large number of bacteriophages.

A.D. Hershey, M. Chase. "Independent Functions of Viral Protein and Nucleic Acid in Growth of Bacteriophage." *Journal of General Physiology* 36 no. 1 (1952):39–56.

Hershey and Chase labeled the protein coat in one batch of phage using radioactive sulfur, ^{35}S , because sulfur is found in the amino acids methionine and cysteine but not in nucleic acids. They labeled the DNA in another batch using radioactive phosphorus, ^{32}P , because phosphorus is found in DNA and RNA but not typically in protein.

Each batch of phage was allowed to infect the cells separately. After infection, Hershey and Chase put each phage bacterial suspension in a blender, which detached the phage coats from the host cell, and spun down the resulting suspension in a centrifuge. The heavier bacterial cells settled down and formed a pellet, whereas the lighter phage particles stayed in the supernatant. In the tube with the protein labeled, the radioactivity remained

only in the supernatant. In the tube with the DNA labeled, the radioactivity was detected only in the bacterial cells. Hershey and Chase concluded that it was the phage DNA that was injected into the cell that carried the information to produce more phage particles, thus proving that DNA, not proteins, was the source of the genetic material. As a result of their work, the scientific community more broadly accepted DNA as the molecule responsible for heredity.



Martha Chase and Alfred Hershey conducted an experiment separately labeling the DNA and proteins of the T2 bacteriophage to determine which component was the genetic material responsible for the production of new phage particles.

By the time Hershey and Chase published their experiment in the early 1950s, microbiologists and other scientists had been researching heredity for over 80 years. Building on one another's research during that time culminated in the general agreement that DNA was the genetic material.

responsible for heredity ([\[link\]](#)). This knowledge set the stage for the age of molecular biology to come and the significant advancements in biotechnology and systems biology that we are experiencing today.

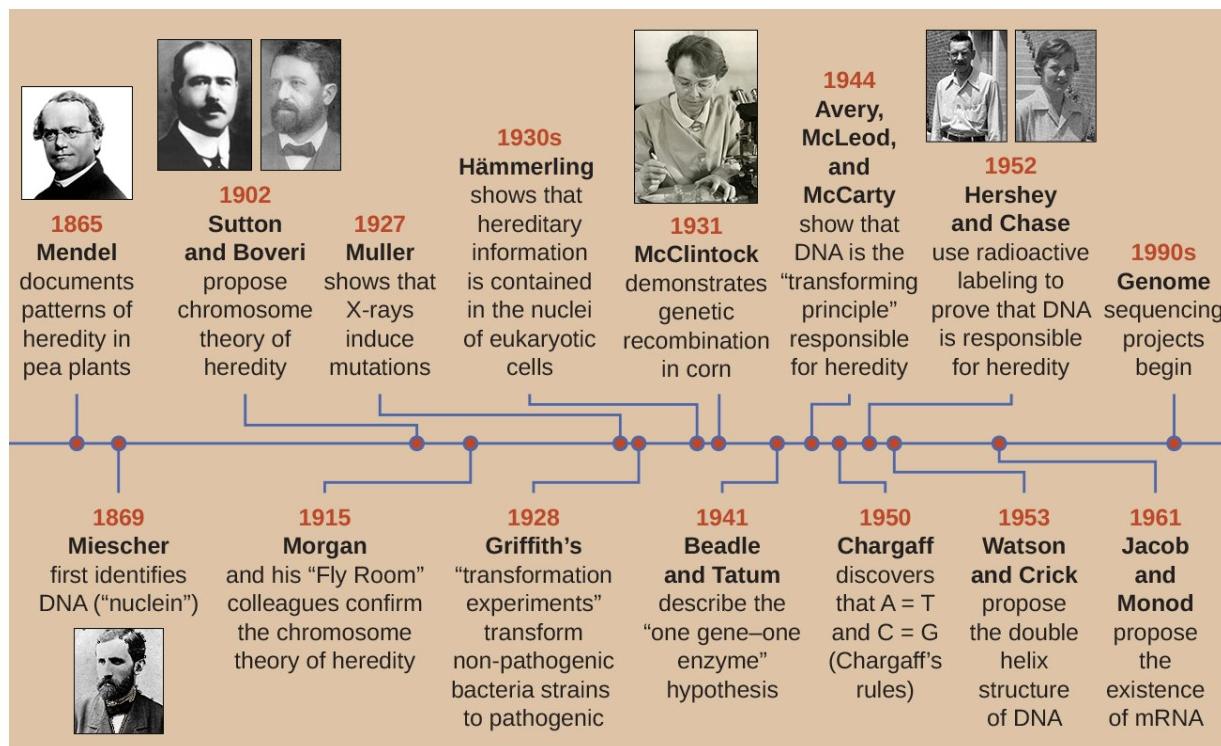
Note:



To learn more about the experiments involved in the history of genetics and the discovery of DNA as the genetic material of cells, visit this [website](#) from the DNA Learning Center.

Note:

- How did Hershey and Chase use microbes to prove that DNA is genetic material?



A timeline of key events leading up to the identification of DNA as the molecule responsible for heredity

Key Concepts and Summary

- **DNA was discovered and characterized long before its role in heredity was understood. Microbiologists played significant roles in demonstrating that DNA is the hereditary information found within cells.**
- In the 1850s and 1860s, Gregor Mendel experimented with true-breeding garden peas to demonstrate the **heritability** of specific observable traits.
- In 1869, Friedrich Miescher isolated and purified a compound rich in phosphorus from the nuclei of white blood cells; he named the compound nuclein. Miescher's student Richard Altmann discovered its acidic nature, renaming it **nucleic acid**. Albrecht Kossel characterized the **nucleotide bases** found within nucleic acids.

- Although Walter Sutton and Theodor Boveri proposed the **Chromosomal Theory of Inheritance** in 1902, it was not scientifically demonstrated until the 1915 publication of the work of Thomas Hunt Morgan and his colleagues.
- Using *Acetabularia*, a large algal cell, as his model system, Joachim Hämerling demonstrated in the 1930s and 1940s that the nucleus was the location of hereditary information in these cells.
- In the 1940s, George Beadle and Edward Tatum used the mold *Neurospora crassa* to show that each protein's production was under the control of a single gene, demonstrating the “**one gene–one enzyme**” hypothesis.
- In 1928, Frederick Griffith showed that dead encapsulated bacteria could pass genetic information to live nonencapsulated bacteria and transform them into harmful strains. In 1944, Oswald Avery, Colin McLeod, and Maclyn McCarty identified the compound as DNA.
- The nature of DNA as the molecule that stores genetic information was unequivocally demonstrated in the experiment of Alfred Hershey and Martha Chase published in 1952. Labeled DNA from bacterial viruses entered and infected bacterial cells, giving rise to more viral particles. The labeled protein coats did not participate in the transmission of genetic information.

Multiple Choice

Exercise:

Problem:

Frederick Griffith infected mice with a combination of dead R and live S bacterial strains. What was the outcome, and why did it occur?

- A. The mice will live. Transformation was not required.
- B. The mice will die. Transformation of genetic material from R to S was required.
- C. The mice will live. Transformation of genetic material from S to R was required.
- D. The mice will die. Transformation was not required.

Solution:

D

Exercise:**Problem:**

Why was the alga *Acetabularia* a good model organism for Joachim Hä默ling to use to identify the location of genetic material?

- A. It lacks a nuclear membrane.
 - B. It self-fertilizes.
 - C. It is a large, asymmetrical, single cell easy to see with the naked eye.
 - D. It makes a protein capsid.
-

Solution:

C

Exercise:**Problem:**

Which of the following best describes the results from Hershey and Chase's experiment using bacterial viruses with ^{35}S -labeled proteins or ^{32}P -labeled DNA that are consistent with protein being the molecule responsible for heredity?

- A. After infection with the ^{35}S -labeled viruses and centrifugation, only the pellet would be radioactive.
- B. After infection with the ^{35}S -labeled viruses and centrifugation, both the pellet and the supernatant would be radioactive.
- C. After infection with the ^{32}P -labeled viruses and centrifugation, only the pellet would be radioactive.
- D. After infection with the ^{32}P -labeled viruses and centrifugation, both the pellet and the supernatant would be radioactive.

Solution:

A

Exercise:**Problem:**

Which method did Morgan and colleagues use to show that hereditary information was carried on chromosomes?

- A. statistical predictions of the outcomes of crosses using true-breeding parents
 - B. correlations between microscopic observations of chromosomal movement and the characteristics of offspring
 - C. transformation of nonpathogenic bacteria to pathogenic bacteria
 - D. mutations resulting in distinct defects in metabolic enzymatic pathways
-

Solution:

B

Exercise:**Problem:**

According to Beadle and Tatum's "one gene–one enzyme" hypothesis, which of the following enzymes will eliminate the transformation of hereditary material from pathogenic bacteria to nonpathogenic bacteria?

- A. carbohydrate-degrading enzymes
- B. proteinases
- C. ribonucleases
- D. deoxyribonucleases

Solution:

D

Fill in the Blank

Exercise:

Problem:

The element _____ is unique to nucleic acids compared with other macromolecules.

Solution:

phosphorus

Exercise:

Problem:

In the late 1800s and early 1900s, the macromolecule thought to be responsible for heredity was _____.

Solution:

protein

Short Answer

Exercise:

Problem:

Why do bacteria and viruses make good model systems for various genetic studies?

Exercise:

Problem:

Why was nucleic acid disregarded for so long as the molecule responsible for the transmission of hereditary information?

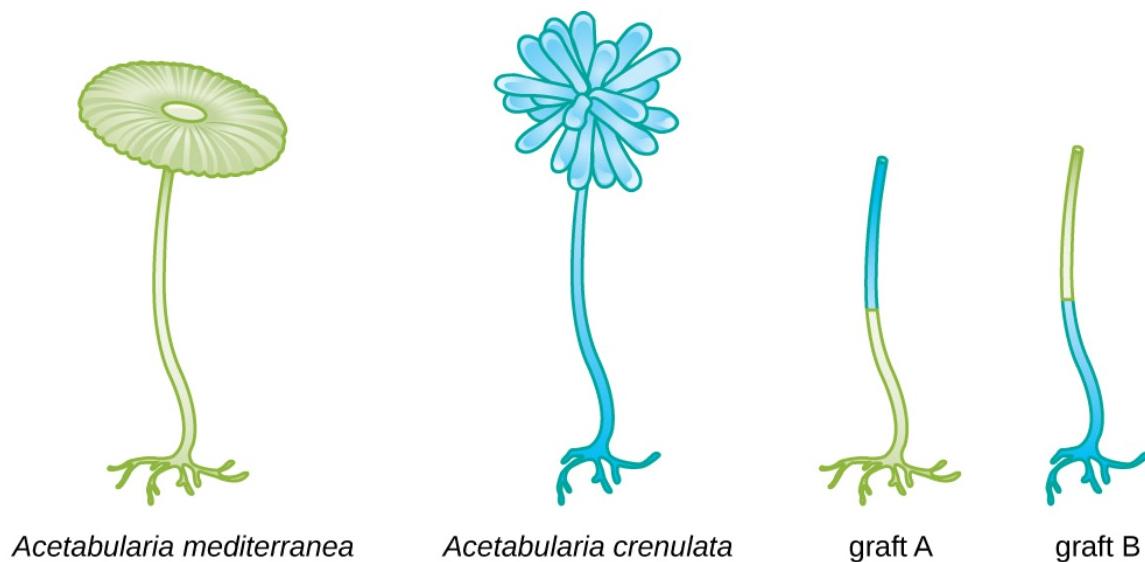
Exercise:**Problem:**

Bacteriophages inject their genetic material into host cells, whereas animal viruses enter host cells completely. Why was it important to use a bacteriophage in the Hershey–Chase experiment rather than an animal virus?

Critical Thinking

Exercise:**Problem:**

In the figure shown, if the nuclei were contained within the stalks of *Acetabularia*, what types of caps would you expect from the pictured grafts?

**Exercise:**

Problem:

Why are Hershey and Chase credited with identifying DNA as the carrier of heredity even though DNA had been discovered many years before?

Structure and Function of DNA

LEARNING OBJECTIVES

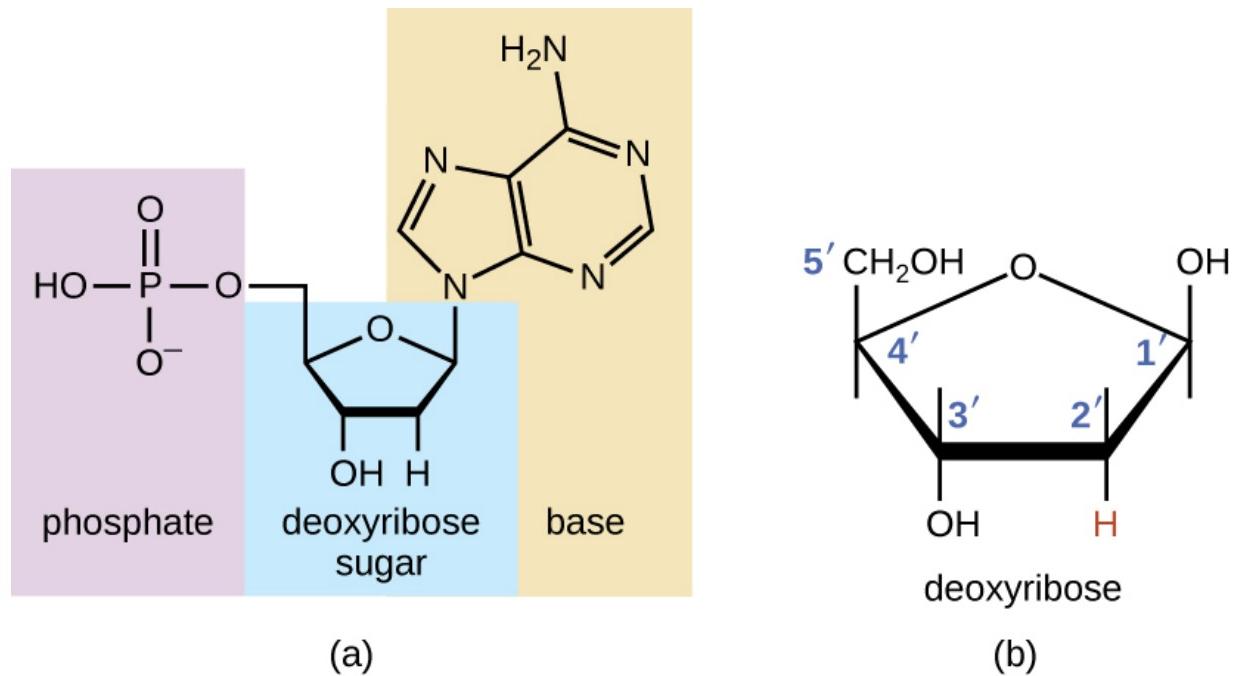
- Describe the biochemical structure of deoxyribonucleotides
- Identify the base pairs used in the synthesis of deoxyribonucleotides
- Explain why the double helix of DNA is described as antiparallel

In [Microbial Metabolism](#), we discussed three classes of macromolecules: proteins, lipids, and carbohydrates. In this chapter, we will discuss a fourth class of macromolecules: nucleic acids. Like other macromolecules, **nucleic acids** are composed of monomers, called **nucleotides**, which are polymerized to form large strands. Each nucleic acid strand contains certain nucleotides that appear in a certain order within the strand, called its **base sequence**. The base sequence of **deoxyribonucleic acid (DNA)** is responsible for carrying and retaining the hereditary information in a cell. In [Mechanisms of Microbial Genetics](#), we will discuss in detail the ways in which DNA uses its own base sequence to direct its own synthesis, as well as the synthesis of RNA and proteins, which, in turn, gives rise to products with diverse structure and function. In this section, we will discuss the basic structure and function of DNA.

DNA Nucleotides

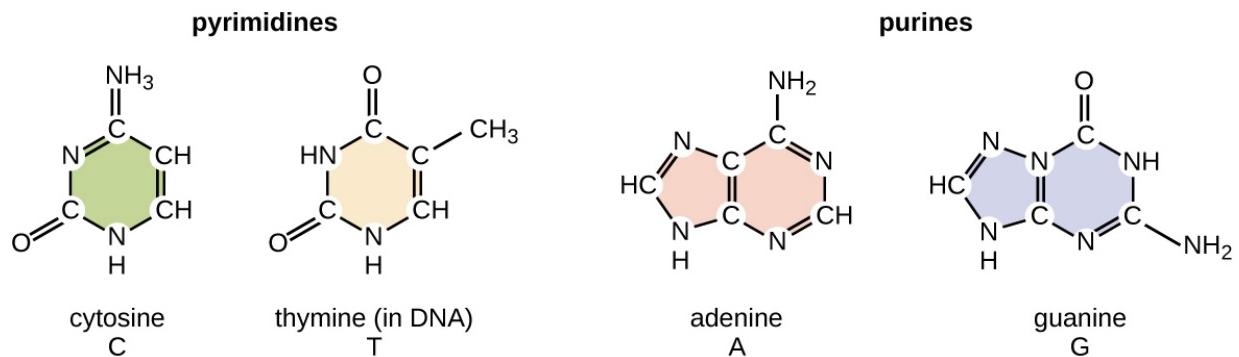
The building blocks of nucleic acids are nucleotides. Nucleotides that compose DNA are called **deoxyribonucleotides**. The three components of a deoxyribonucleotide are a five-carbon sugar called deoxyribose, a

phosphate group, and a **nitrogenous base**, a nitrogen-containing ring structure that is responsible for complementary base pairing between nucleic acid strands ([\[link\]](#)). The carbon atoms of the five-carbon deoxyribose are numbered 1', 2', 3', 4', and 5' (1' is read as “one prime”). A nucleoside comprises the five-carbon sugar and nitrogenous base.



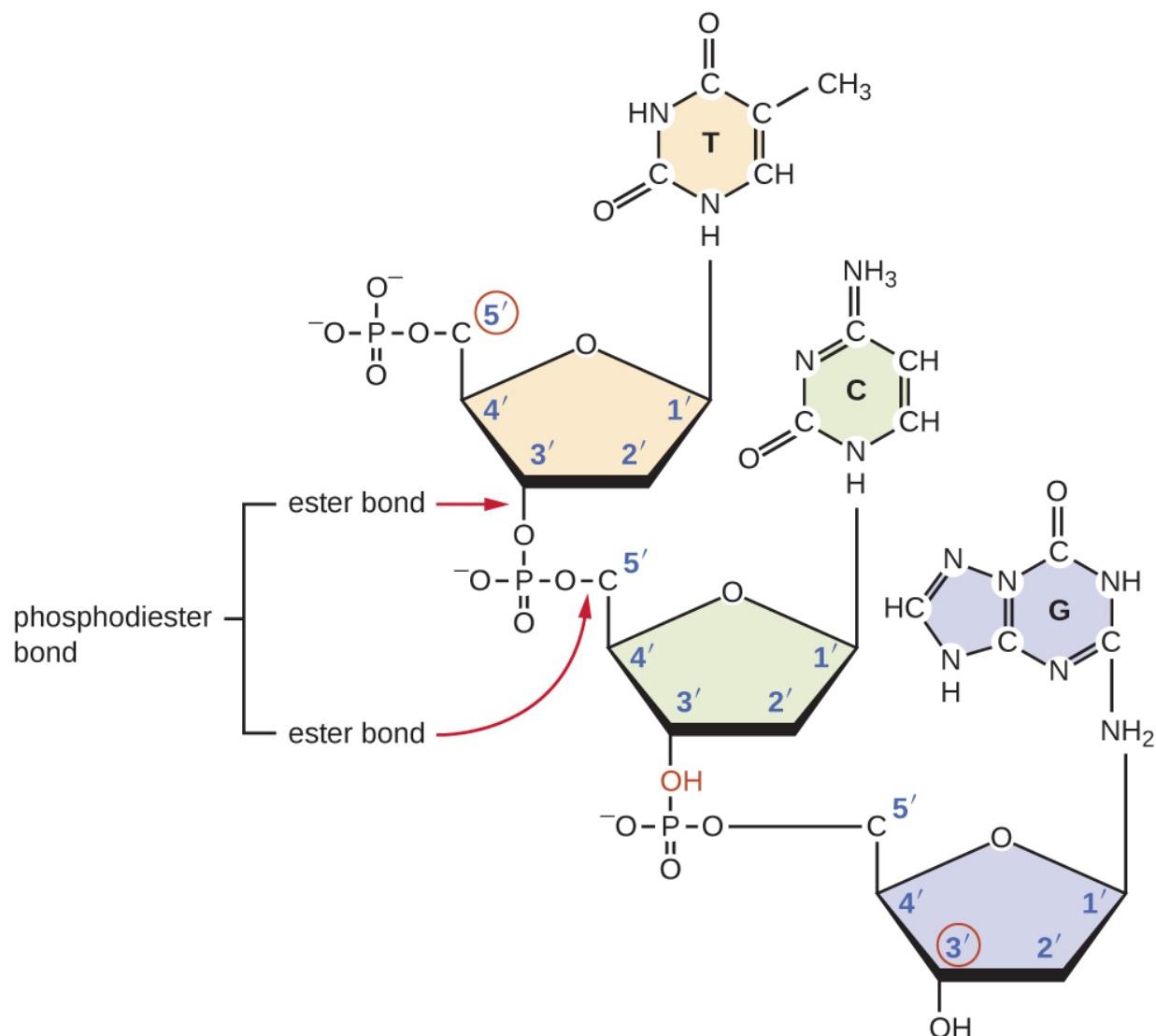
(a) Each deoxyribonucleotide is made up of a sugar called deoxyribose, a phosphate group, and a nitrogenous base—in this case, adenine. (b) The five carbons within deoxyribose are designated as 1', 2', 3', 4', and 5'.

The deoxyribonucleotide is named according to the nitrogenous bases ([\[link\]](#)). The nitrogenous bases **adenine** (A) and **guanine** (G) are the **purines**; they have a double-ring structure with a six-carbon ring fused to a five-carbon ring. The **pyrimidines**, **cytosine** (C) and **thymine** (T), are smaller nitrogenous bases that have only a six-carbon ring structure.



Nitrogenous bases within DNA are categorized into the two-ringed purines adenine and guanine and the single-ringed pyrimidines cytosine and thymine. Thymine is unique to DNA.

Individual nucleoside triphosphates combine with each other by covalent bonds known as **5'-3' phosphodiester bonds**, or linkages whereby the phosphate group attached to the 5' carbon of the sugar of one nucleotide bonds to the hydroxyl group of the 3' carbon of the sugar of the next nucleotide. Phosphodiester bonding between nucleotides forms the **sugar-phosphate backbone**, the alternating sugar-phosphate structure composing the framework of a nucleic acid strand ([\[link\]](#)). During the polymerization process, deoxynucleotide triphosphates (dNTP) are used. To construct the sugar-phosphate backbone, the two terminal phosphates are released from the dNTP as a pyrophosphate. The resulting strand of nucleic acid has a free phosphate group at the 5' carbon end and a free hydroxyl group at the 3' carbon end. The two unused phosphate groups from the nucleotide triphosphate are released as pyrophosphate during phosphodiester bond formation. Pyrophosphate is subsequently hydrolyzed, releasing the energy used to drive nucleotide polymerization.



Phosphodiester bonds form between the phosphate group attached to the 5' carbon of one nucleotide and the hydroxyl group of the 3' carbon in the next nucleotide, bringing about polymerization of nucleotides into nucleic acid strands. Note the 5' and 3' ends of this nucleic acid strand.

Note:

- What is meant by the 5' and 3' ends of a nucleic acid strand?

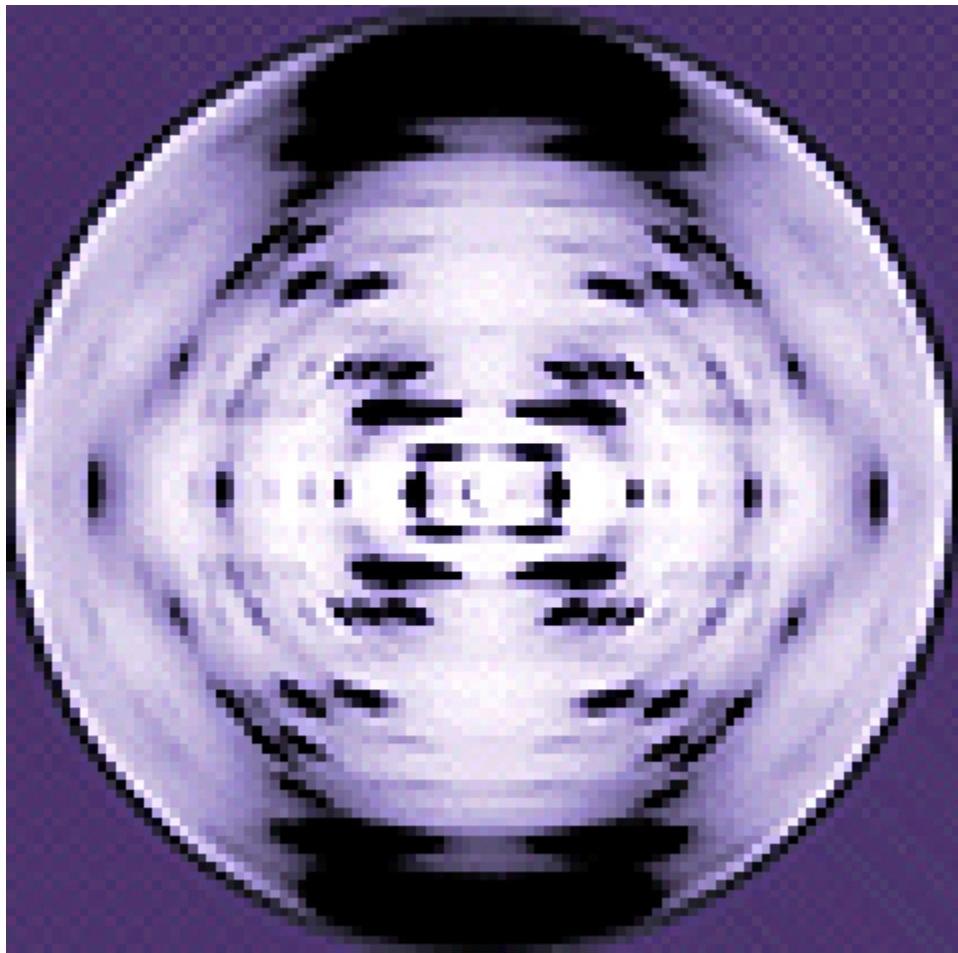
Discovering the Double Helix

By the early 1950s, considerable evidence had accumulated indicating that DNA was the genetic material of cells, and now the race was on to discover its three-dimensional structure. Around this time, Austrian biochemist Erwin Chargaff [[footnote](#)] (1905–2002) examined the content of DNA in different species and discovered that adenine, thymine, guanine, and cytosine were not found in equal quantities, and that it varied from species to species, but not between individuals of the same species. He found that the amount of adenine was very close to equaling the amount of thymine, and the amount of cytosine was very close to equaling the amount of guanine, or A = T and G = C. These relationships are also known as Chargaff's rules.

N. Kresge et al. "Chargaff's Rules: The Work of Erwin Chargaff." *Journal of Biological Chemistry* 280 (2005):e21.

Other scientists were also actively exploring this field during the mid-20th century. In 1952, American scientist Linus Pauling (1901–1994) was the world's leading structural chemist and odds-on favorite to solve the structure of DNA. Pauling had earlier discovered the structure of protein α helices, using X-ray diffraction, and, based upon X-ray diffraction images of DNA made in his laboratory, he proposed a triple-stranded model of DNA. [[footnote](#)] At the same time, British researchers Rosalind Franklin (1920–1958) and her graduate student R.G. Gosling were also using X-ray diffraction to understand the structure of DNA ([\[link\]](#)). It was Franklin's scientific expertise that resulted in the production of more well-defined X-ray diffraction images of DNA that would clearly show the overall double-helix structure of DNA.

L. Pauling, "A Proposed Structure for the Nucleic Acids." *Proceedings of the National Academy of Science of the United States of America* 39 no. 2 (1953):84–97.



The X-ray diffraction pattern of DNA shows its helical nature. (credit: National Institutes of Health)

James Watson (1928–), an American scientist, and Francis Crick (1916–2004), a British scientist, were working together in the 1950s to discover DNA’s structure. They used Chargaff’s rules and Franklin and Wilkins’ X-ray diffraction images of DNA fibers to piece together the purine-pyrimidine pairing of the double helical DNA molecule ([\[link\]](#)). In April 1953, Watson and Crick published their model of the DNA double helix in *Nature*.[\[footnote\]](#) The same issue additionally included papers by Wilkins and colleagues,[\[footnote\]](#) as well as by Franklin and Gosling,[\[footnote\]](#) each describing different aspects of the molecular structure of DNA. In 1962, James Watson, Francis Crick, and Maurice Wilkins were awarded the

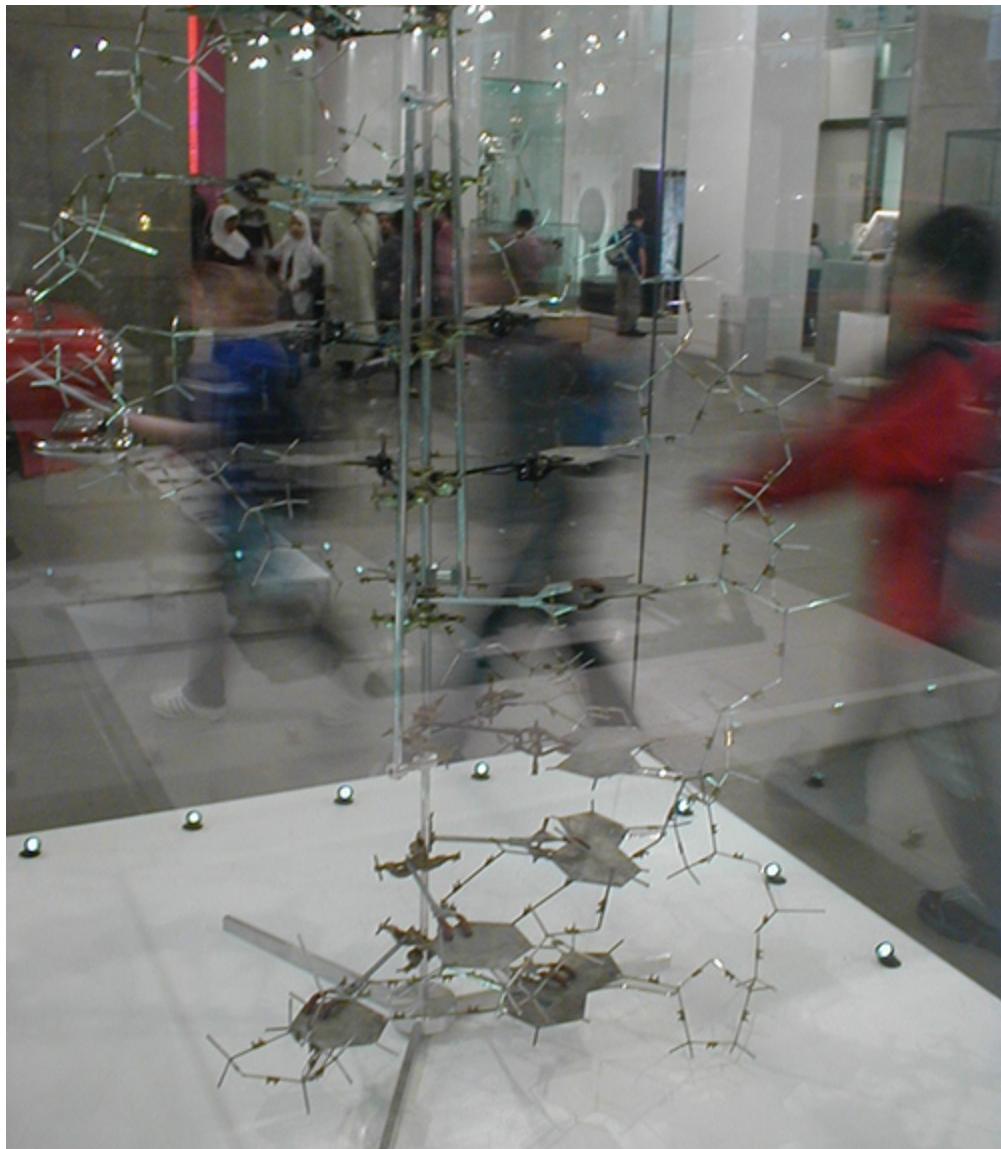
Nobel Prize in Physiology and Medicine. Unfortunately, by then Franklin had died, and Nobel prizes at the time were not awarded posthumously. Work continued, however, on learning about the structure of DNA. In 1973, Alexander Rich (1924–2015) and colleagues were able to analyze DNA crystals to confirm and further elucidate DNA structure.[\[footnote\]](#)

J.D. Watson, F.H.C. Crick. “A Structure for Deoxyribose Nucleic Acid.” *Nature* 171 no. 4356 (1953):737–738.

M.H.F. Wilkins et al. “Molecular Structure of Deoxypentose Nucleic Acids.” *Nature* 171 no. 4356 (1953):738–740.

R. Franklin, R.G. Gosling. “Molecular Configuration in Sodium Thymonucleate.” *Nature* 171 no. 4356 (1953):740–741.

R.O. Day et al. “A Crystalline Fragment of the Double Helix: The Structure of the Dinucleoside Phosphate Guanylyl-3',5'-Cytidine.” *Proceedings of the National Academy of Sciences of the United States of America* 70 no. 3 (1973):849–853.



In 1953, James Watson and Francis Crick built this model of the structure of DNA, shown here on display at the Science Museum in London.

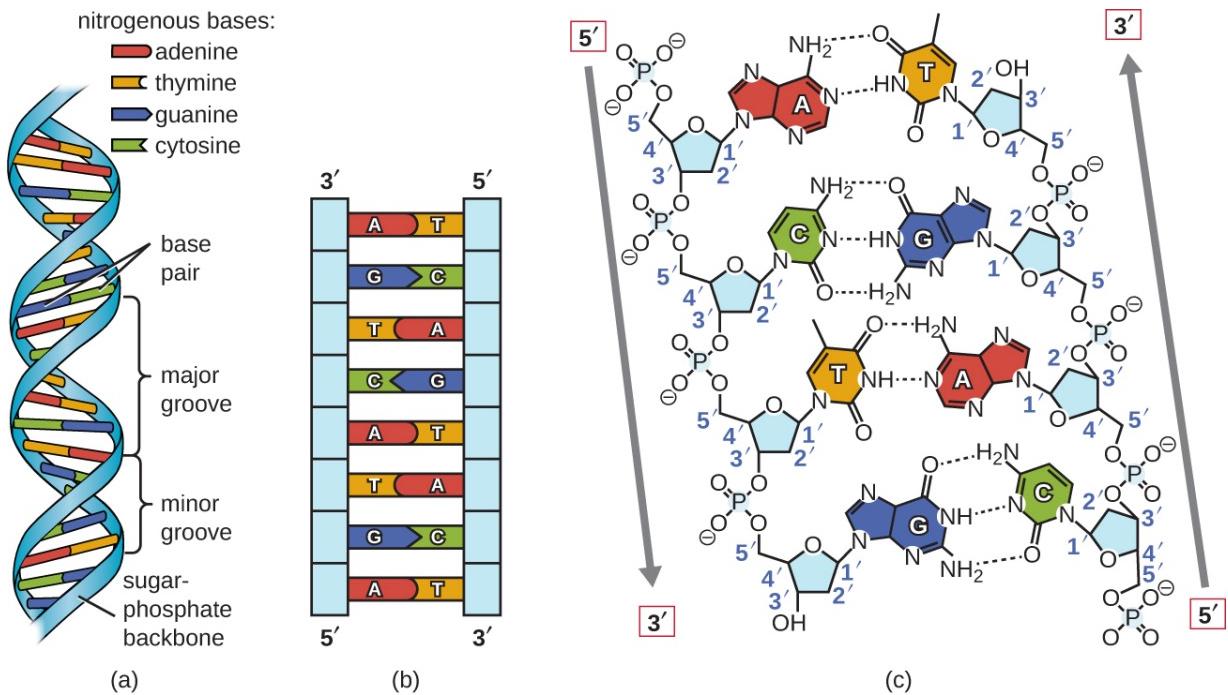
Note:

- Which scientists are given most of the credit for describing the molecular structure of DNA?

DNA Structure

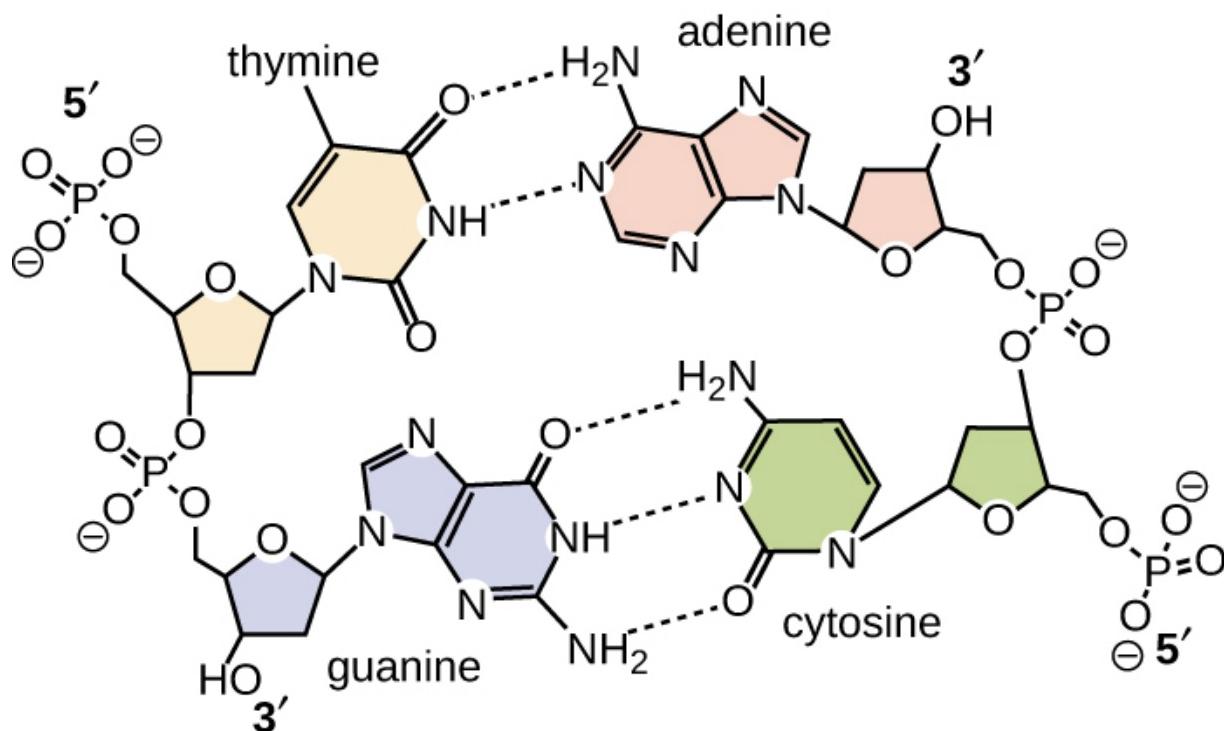
Watson and Crick proposed that DNA is made up of two strands that are twisted around each other to form a right-handed helix. The two DNA strands are **antiparallel**, such that the 3' end of one strand faces the 5' end of the other ([\[link\]](#)). The 3' end of each strand has a free hydroxyl group, while the 5' end of each strand has a free phosphate group. The sugar and phosphate of the polymerized nucleotides form the backbone of the structure, whereas the nitrogenous bases are stacked inside. These nitrogenous bases on the interior of the molecule interact with each other, base pairing.

Analysis of the diffraction patterns of DNA has determined that there are approximately 10 bases per turn in DNA. The asymmetrical spacing of the sugar-phosphate backbones generates major grooves (where the backbone is far apart) and minor grooves (where the backbone is close together) ([\[link\]](#)). These grooves are locations where proteins can bind to DNA. The binding of these proteins can alter the structure of DNA, regulate replication, or regulate transcription of DNA into RNA.



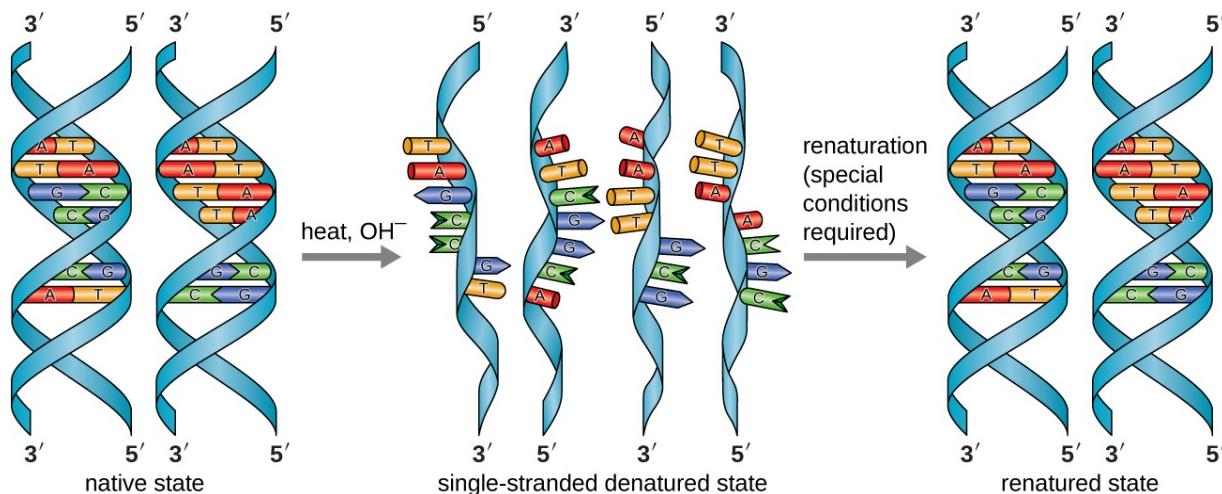
Watson and Crick proposed the double helix model for DNA. (a) The sugar-phosphate backbones are on the outside of the double helix and purines and pyrimidines form the “rungs” of the DNA helix ladder. (b) The two DNA strands are antiparallel to each other. (c) The direction of each strand is identified by numbering the carbons (1 through 5) in each sugar molecule. The 5' end is the one where carbon #5 is not bound to another nucleotide; the 3' end is the one where carbon #3 is not bound to another nucleotide.

Base pairing takes place between a purine and pyrimidine. In DNA, adenine (A) and thymine (T) are **complementary base pairs**, and cytosine (C) and guanine (G) are also complementary base pairs, explaining Chargaff’s rules ([\[link\]](#)). The base pairs are stabilized by hydrogen bonds; adenine and thymine form two hydrogen bonds between them, whereas cytosine and guanine form three hydrogen bonds between them.



Hydrogen bonds form between complementary nitrogenous bases on the interior of DNA.

In the laboratory, exposing the two DNA strands of the double helix to high temperatures or to certain chemicals can break the hydrogen bonds between complementary bases, thus separating the strands into two separate single strands of DNA (single-stranded DNA [ssDNA]). This process is called DNA denaturation and is analogous to protein denaturation, as described in [Proteins](#). The ssDNA strands can also be put back together as double-stranded DNA (dsDNA), through reannealing or renaturing by cooling or removing the chemical denaturants, allowing these hydrogen bonds to reform. The ability to artificially manipulate DNA in this way is the basis for several important techniques in biotechnology ([\[link\]](#)). Because of the additional hydrogen bonding between the C = G base pair, DNA with a high GC content is more difficult to denature than DNA with a lower GC content.



In the laboratory, the double helix can be denatured to single-stranded DNA through exposure to heat or chemicals, and then renatured through cooling or removal of chemical denaturants to allow the DNA strands to reanneal. (credit: modification of work by Hernández-Lemus E, Nicasio-Collazo LA, Castañeda-Priego R)

Note:



View an [animation](#) on DNA structure from the DNA Learning Center to learn more.

Note:

- What are the two complementary base pairs of DNA and how are they bonded together?

DNA Function

DNA stores the information needed to build and control the cell. The transmission of this information from mother to daughter cells is called **vertical gene transfer** and it occurs through the process of DNA replication. DNA is replicated when a cell makes a duplicate copy of its DNA, then the cell divides, resulting in the correct distribution of one DNA copy to each resulting cell. DNA can also be enzymatically degraded and used as a source of nucleosides and nucleotides for the cell. Unlike other macromolecules, DNA does not serve a structural role in cells.

Note:

- How does DNA transmit genetic information to offspring?

Note:

Paving the Way for Women in Science and Health Professions

Historically, women have been underrepresented in the sciences and in medicine, and often their pioneering contributions have gone relatively unnoticed. For example, although Rosalind Franklin performed the X-ray diffraction studies demonstrating the double helical structure of DNA, it is Watson and Crick who became famous for this discovery, building on her data. There still remains great controversy over whether their acquisition of her data was appropriate and whether personality conflicts and gender bias contributed to the delayed recognition of her significant contributions. Similarly, Barbara McClintock did pioneering work in maize (corn) genetics from the 1930s through 1950s, discovering transposons (jumping

genes), but she was not recognized until much later, receiving a Nobel Prize in Physiology or Medicine in 1983 ([\[link\]](#)).

Today, women still remain underrepresented in many fields of science and medicine. While more than half of the undergraduate degrees in science are awarded to women, only 46% of doctoral degrees in science are awarded to women. In academia, the number of women at each level of career advancement continues to decrease, with women holding less than one-third of the positions of Ph.D.-level scientists in tenure-track positions, and less than one-quarter of the full professorships at 4-year colleges and universities.[\[footnote\]](#) Even in the health professions, like nearly all other fields, women are often underrepresented in many medical careers and earn significantly less than their male counterparts, as shown in a 2013 study published by the *Journal of the American Medical Association*.[\[footnote\]](#) N.H. Wolfinger “For Female Scientists, There's No Good Time to Have Children.” *The Atlantic* July 29, 2013.

<http://www.theatlantic.com/sexes/archive/2013/07/for-female-scientists-theres-no-good-time-to-have-children/278165/>.

S.A. Seabury et al. “Trends in the Earnings of Male and Female Health Care Professionals in the United States, 1987 to 2010.” *Journal of the American Medical Association Internal Medicine* 173 no. 18 (2013):1748–1750.

Why do such disparities continue to exist and how do we break these cycles? The situation is complex and likely results from the combination of various factors, including how society conditions the behaviors of girls from a young age and supports their interests, both professionally and personally. Some have suggested that women do not belong in the laboratory, including Nobel Prize winner Tim Hunt, whose 2015 public comments suggesting that women are too emotional for science[\[footnote\]](#) were met with widespread condemnation.

E. Chung. “Tim Hunt, Sexism and Science: The Real 'Trouble With Girls' in Labs.” *CBC News Technology and Science*, June 12, 2015.

<http://www.cbc.ca/news/technology/tim-hunt-sexism-and-science-the-real-trouble-with-girls-in-labs-1.3110133>. Accessed 8/4/2016.

Perhaps girls should be supported more from a young age in the areas of science and math ([\[link\]](#)). Science, technology, engineering, and mathematics (STEM) programs sponsored by the American Association of University Women (AAUW)[\[footnote\]](#) and National Aeronautics and

Space Administration (NASA)[\[footnote\]](#) are excellent examples of programs that offer such support. Contributions by women in science should be made known more widely to the public, and marketing targeted to young girls should include more images of historically and professionally successful female scientists and medical professionals, encouraging all bright young minds, including girls and women, to pursue careers in science and medicine.

American Association of University Women. “Building a STEM Pipeline for Girls and Women.” <http://www.aauw.org/what-we-do/stem-education/>. Accessed June 10, 2016.

National Aeronautics and Space Administration. “Outreach Programs: Women and Girls Initiative.” <http://women.nasa.gov/outreach-programs/>. Accessed June 10, 2016.



(a)



(b)

(a) Barbara McClintock’s work on maize genetics in the 1930s through 1950s resulted in the discovery of transposons, but its significance was not recognized at the time. (b) Efforts to appropriately mentor and to provide continued societal support for women in science and medicine may someday help alleviate some of the issues preventing gender equality at all levels in science and medicine. (credit a: modification of work by Smithsonian Institution; credit b: modification of work by Haynie SL, Hinkle AS, Jones NL, Martin CA, Olsiewski PJ, Roberts MF)

Key Concepts and Summary

- Nucleic acids are composed of **nucleotides**, each of which contains a pentose sugar, a phosphate group, and a **nitrogenous base**. **Deoxyribonucleotides** within DNA contain **deoxyribose** as the pentose sugar.
- DNA contains the **pyrimidines cytosine** and **thymine**, and the **purines adenine** and **guanine**.
- **Nucleotides** are linked together by phosphodiester bonds between the 5' phosphate group of one nucleotide and the 3' hydroxyl group of another. A **nucleic acid strand** has a free phosphate group at the 5' end and a free hydroxyl group at the 3' end.
- Chargaff discovered that the amount of **adenine** is approximately equal to the amount of **thymine** in DNA, and that the amount of the **guanine** is approximately equal to **cytosine**. These relationships were later determined to be due to complementary base pairing.
- Watson and Crick, building on the work of Chargaff, Franklin and Gosling, and Wilkins, proposed the double helix model and base pairing for DNA structure.
- DNA is composed of two complementary strands oriented **antiparallel** to each other with the **phosphodiester backbones** on the exterior of the molecule. The nitrogenous bases of each strand face each other and complementary bases hydrogen bond to each other, stabilizing the double helix.
- Heat or chemicals can break the hydrogen bonds between complementary bases, denaturing DNA. Cooling or removing chemicals can lead to renaturation or reannealing of DNA by allowing hydrogen bonds to reform between complementary bases.
- DNA stores the instructions needed to build and control the cell. This information is transmitted from parent to offspring through **vertical gene transfer**.

Critical Thinking

Exercise:

Problem:

A certain DNA sample is found to have a makeup consisting of 22% thymine. Use Chargaff's rules to fill in the percentages for the other three nitrogenous bases.

adenine	guanine	thymine	cytosine
___%	___%	22%	___%

Exercise:

Problem:

In considering the structure of the DNA double helix, how would you expect the structure to differ if there was base pairing between two purines? Between two pyrimidines?

Structure and Function of RNA

LEARNING OBJECTIVES

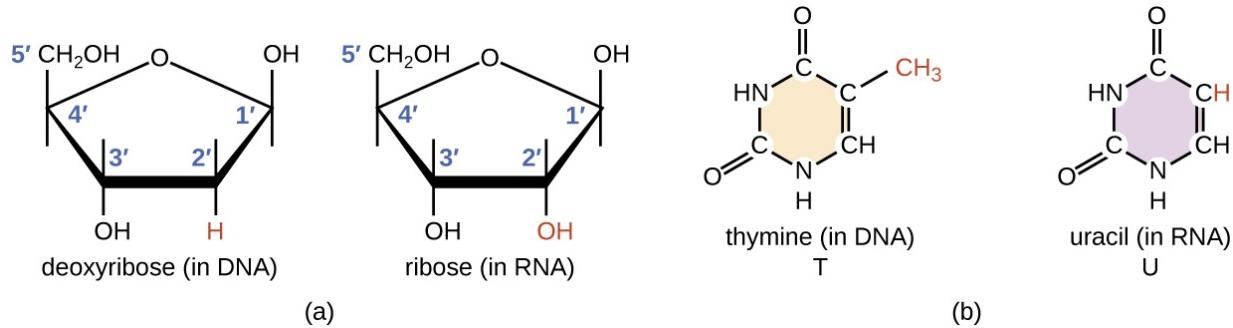
- Describe the biochemical structure of ribonucleotides
- Describe the similarities and differences between RNA and DNA
- Describe the functions of the three main types of RNA used in protein synthesis
- Explain how RNA can serve as hereditary information

Structurally speaking, **ribonucleic acid (RNA)**, is quite similar to DNA. However, whereas DNA molecules are typically long and double stranded, RNA molecules are much shorter and are typically single stranded. RNA molecules perform a variety of roles in the cell but are mainly involved in the process of protein synthesis (translation) and its regulation.

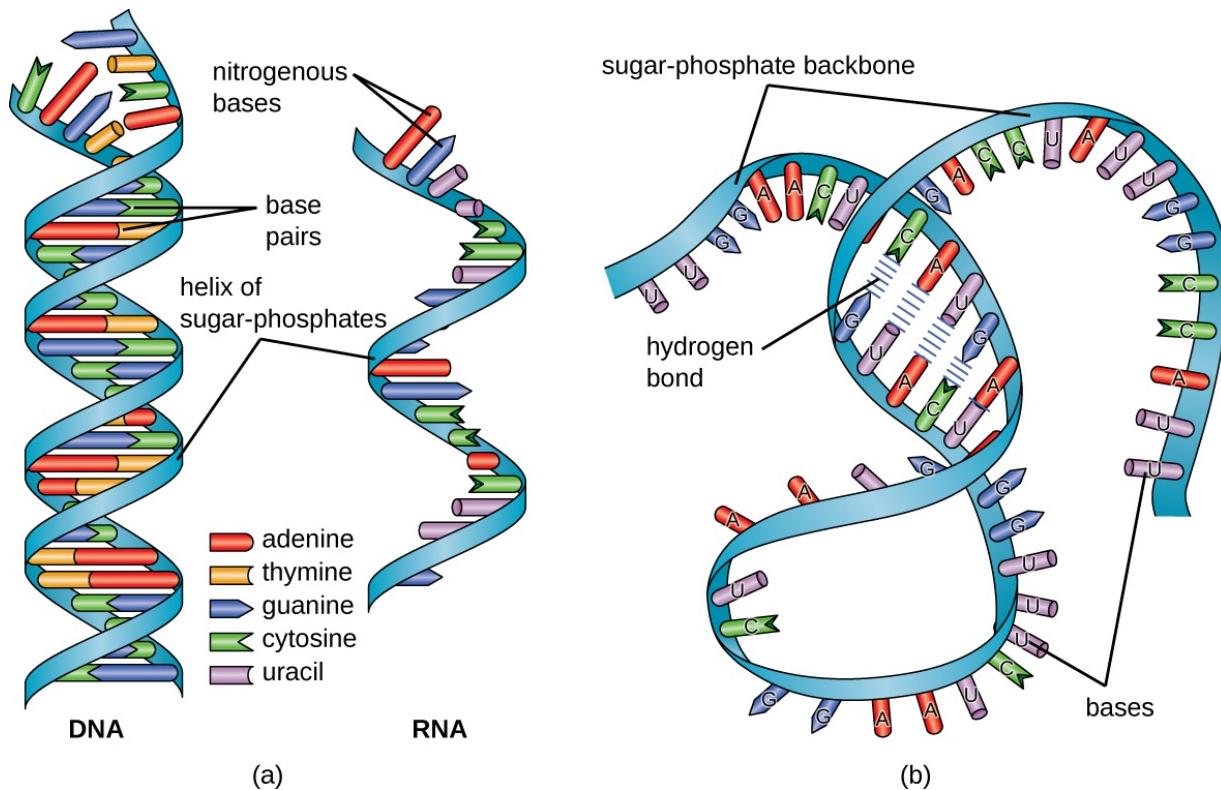
RNA Structure

RNA is typically single stranded and is made of **ribonucleotides** that are linked by phosphodiester bonds. A ribonucleotide in the RNA chain contains ribose (the pentose sugar), one of the four nitrogenous bases (A, U, G, and C), and a phosphate group. The subtle structural difference between the sugars gives DNA added stability, making DNA more suitable for storage of genetic information, whereas the relative instability of RNA makes it more suitable for its more short-term functions. The RNA-specific pyrimidine **uracil** forms a complementary base pair with adenine and is used instead of the thymine used in DNA. Even though RNA is single stranded, most types of RNA molecules show extensive intramolecular base

pairing between complementary sequences within the RNA strand, creating a predictable three-dimensional structure essential for their function ([\[link\]](#) and [\[link\]](#)).



(a) Ribonucleotides contain the pentose sugar ribose instead of the deoxyribose found in deoxyribonucleotides. (b) RNA contains the pyrimidine uracil in place of thymine found in DNA.



(a) DNA is typically double stranded, whereas RNA is typically single stranded. (b) Although it is single stranded, RNA can fold upon itself, with the folds stabilized by short areas of complementary base pairing within the molecule, forming a three-dimensional structure.

Note:

- How does the structure of RNA differ from the structure of DNA?

Functions of RNA in Protein Synthesis

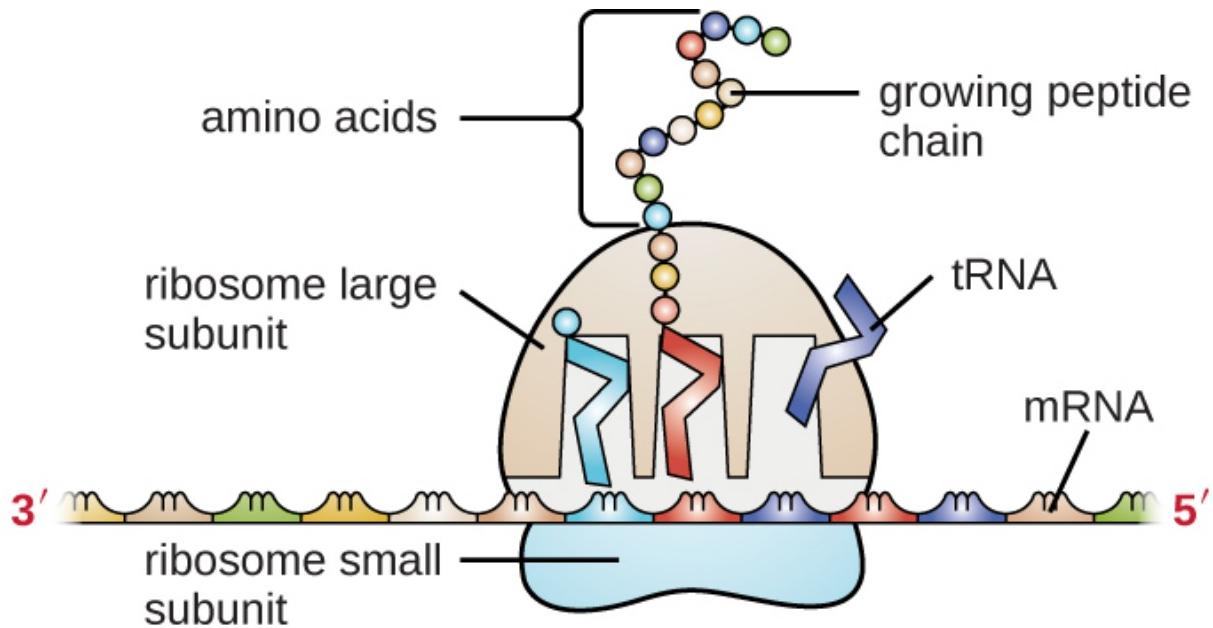
Cells access the information stored in DNA by creating RNA to direct the synthesis of proteins through the process of translation. Proteins within a

cell have many functions, including building cellular structures and serving as enzyme catalysts for cellular chemical reactions that give cells their specific characteristics. The three main types of RNA directly involved in protein synthesis are **messenger RNA (mRNA)**, **ribosomal RNA (rRNA)**, and **transfer RNA (tRNA)**.

In 1961, French scientists François Jacob and Jacques Monod hypothesized the existence of an intermediary between DNA and its protein products, which they called messenger RNA.[\[footnote\]](#) Evidence supporting their hypothesis was gathered soon afterwards showing that information from DNA is transmitted to the ribosome for protein synthesis using mRNA. If DNA serves as the complete library of cellular information, mRNA serves as a photocopy of specific information needed at a particular point in time that serves as the instructions to make a protein.

A. Rich. “The Era of RNA Awakening: Structural Biology of RNA in the Early Years.” *Quarterly Reviews of Biophysics* 42 no. 2 (2009):117–137.

The mRNA carries the message from the DNA, which controls all of the cellular activities in a cell. If a cell requires a certain protein to be synthesized, the gene for this product is “turned on” and the mRNA is synthesized through the process of transcription (see [RNA Transcription](#)). The mRNA then interacts with ribosomes and other cellular machinery ([\[link\]](#)) to direct the synthesis of the protein it encodes during the process of translation (see [Protein Synthesis](#)). mRNA is relatively unstable and short-lived in the cell, especially in prokaryotic cells, ensuring that proteins are only made when needed.



A generalized illustration of how mRNA and tRNA are used in protein synthesis within a cell.

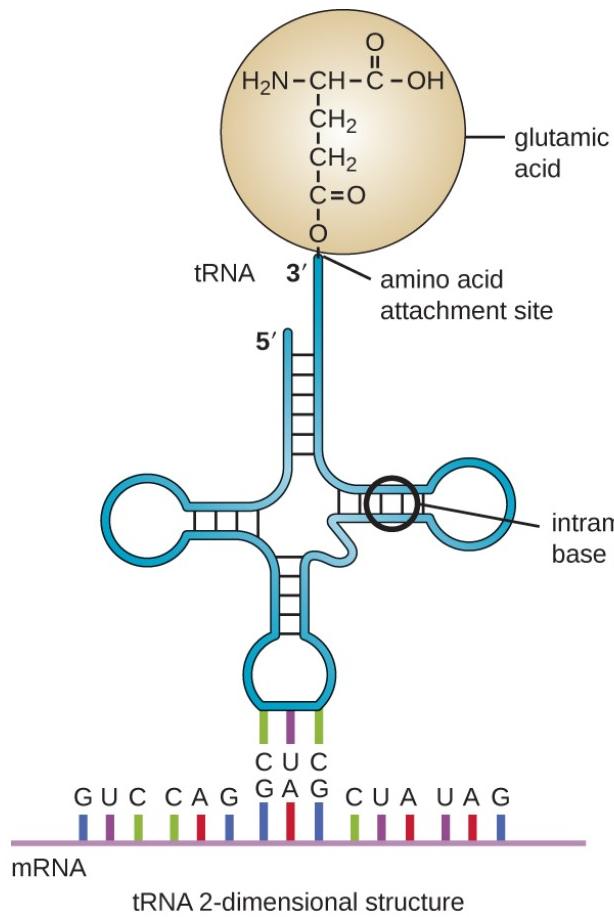
rRNA and tRNA are stable types of RNA. In prokaryotes and eukaryotes, tRNA and rRNA are encoded in the DNA, then copied into long RNA molecules that are cut to release smaller fragments containing the individual mature RNA species. In eukaryotes, synthesis, cutting, and assembly of rRNA into ribosomes takes place in the nucleolus region of the nucleus, but these activities occur in the cytoplasm of prokaryotes. Neither of these types of RNA carries instructions to direct the synthesis of a polypeptide, but they play other important roles in protein synthesis.

Ribosomes are composed of rRNA and protein. As its name suggests, rRNA is a major constituent of ribosomes, composing up to about 60% of the ribosome by mass and providing the location where the mRNA binds. The rRNA ensures the proper alignment of the mRNA, tRNA, and the ribosomes; the rRNA of the ribosome also has an enzymatic activity (peptidyl transferase) and catalyzes the formation of the peptide bonds between two aligned amino acids during protein synthesis. Although rRNA had long been thought to serve primarily a structural role, its catalytic role

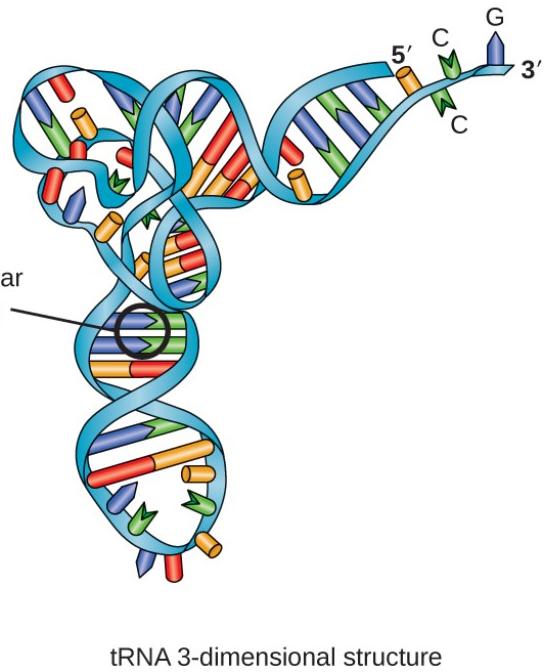
within the ribosome was proven in 2000.[\[footnote\]](#) Scientists in the laboratories of Thomas Steitz (1940–) and Peter Moore (1939–) at Yale University were able to crystallize the ribosome structure from *Haloarcula marismortui*, a halophilic archaeon isolated from the Dead Sea. Because of the importance of this work, Steitz shared the 2009 Nobel Prize in Chemistry with other scientists who made significant contributions to the understanding of ribosome structure.

P. Nissen et al. “The Structural Basis of Ribosome Activity in Peptide Bond Synthesis.” *Science* 289 no. 5481 (2000):920–930.

Transfer RNA is the third main type of RNA and one of the smallest, usually only 70–90 nucleotides long. It carries the correct amino acid to the site of protein synthesis in the ribosome. It is the base pairing between the tRNA and mRNA that allows for the correct amino acid to be inserted in the polypeptide chain being synthesized ([\[link\]](#)). Any mutations in the tRNA or rRNA can result in global problems for the cell because both are necessary for proper protein synthesis ([\[link\]](#)).



tRNA 2-dimensional structure



tRNA 3-dimensional structure

A tRNA molecule is a single-stranded molecule that exhibits significant intracellular base pairing, giving it its characteristic three-dimensional shape.

Structure and Function of RNA

	mRNA	rRNA	tRNA
--	-------------	-------------	-------------

Structure and Function of RNA

	mRNA	rRNA	tRNA
Structure	Short, unstable, single-stranded RNA corresponding to a gene encoded within DNA	Longer, stable RNA molecules composing 60% of ribosome's mass	Short (70-90 nucleotides), stable RNA with extensive intramolecular base pairing; contains an amino acid binding site and an mRNA binding site
Function	Serves as intermediary between DNA and protein; used by ribosome to direct synthesis of protein it encodes	Ensures the proper alignment of mRNA, tRNA, and ribosome during protein synthesis; catalyzes peptide bond formation between amino acids	Carries the correct amino acid to the site of protein synthesis in the ribosome

Note:

- What are the functions of the three major types of RNA molecules involved in protein synthesis?

RNA as Hereditary Information

Although RNA does not serve as the hereditary information in most cells, RNA does hold this function for many viruses that do not contain DNA. Thus, RNA clearly does have the additional capacity to serve as genetic information. Although RNA is typically single stranded within cells, there is significant diversity in viruses. Rhinoviruses, which cause the common cold; influenza viruses; and the Ebola virus are single-stranded RNA viruses. Rotaviruses, which cause severe gastroenteritis in children and other immunocompromised individuals, are examples of double-stranded RNA viruses. Because double-stranded RNA is uncommon in eukaryotic cells, its presence serves as an indicator of viral infection. The implications for a virus having an RNA genome instead of a DNA genome are discussed in more detail in [Viruses](#).

Key Concepts and Summary

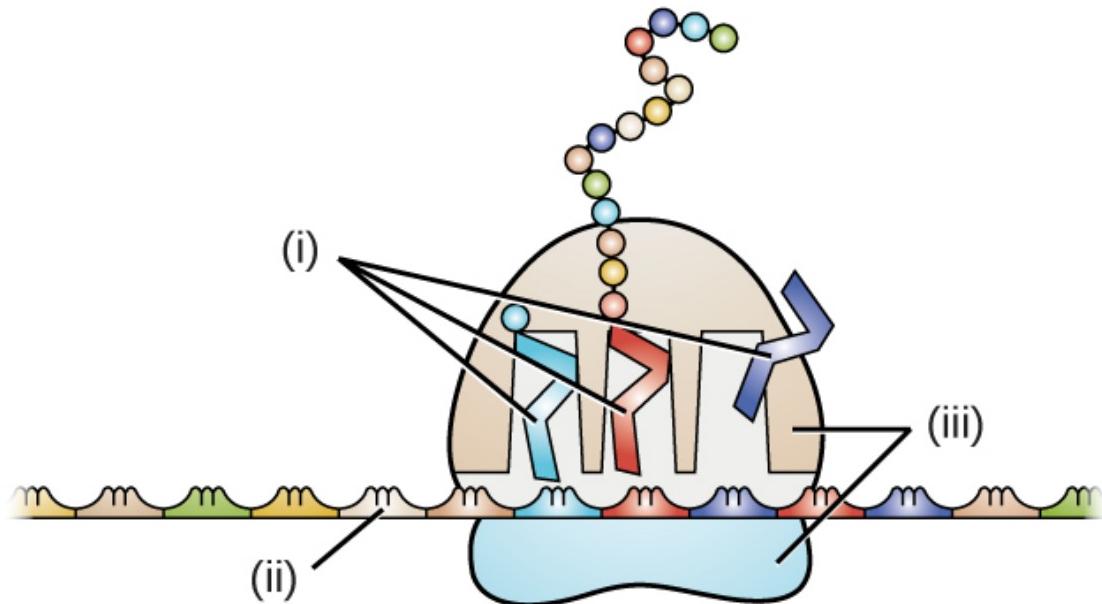
- **Ribonucleic acid (RNA)** is typically single stranded and contains ribose as its pentose sugar and the pyrimidine uracil instead of thymine. An RNA strand can undergo significant intramolecular base pairing to take on a three-dimensional structure.
- There are three main types of RNA, all involved in protein synthesis.
- Messenger RNA (**mRNA**) serves as the intermediary between DNA and the synthesis of protein products during translation.
- Ribosomal RNA (**rRNA**) is a type of stable RNA that is a major constituent of ribosomes. It ensures the proper alignment of the mRNA and the ribosomes during protein synthesis and catalyzes the formation of the peptide bonds between two aligned amino acids during protein synthesis.
- Transfer RNA (**tRNA**) is a small type of stable RNA that carries an amino acid to the corresponding site of protein synthesis in the ribosome. It is the base pairing between the tRNA and mRNA that allows for the correct amino acid to be inserted in the polypeptide chain being synthesized.
- Although RNA is not used for long-term genetic information in cells, many viruses do use RNA as their genetic material.

Critical Thinking

Exercise:

Problem:

Identify the location of mRNA, rRNA, and tRNA in the figure.



Exercise:

Problem:

Why does it make sense that tRNA and rRNA molecules are more stable than mRNA molecules?

Structure and Function of Cellular Genomes

LEARNING OBJECTIVES

- Define gene and genotype and differentiate genotype from phenotype
- Describe chromosome structure and packaging
- Compare prokaryotic and eukaryotic chromosomes
- Explain why extrachromosomal DNA is important in a cell

Thus far, we have discussed the structure and function of individual pieces of DNA and RNA. In this section, we will discuss how all of an organism's genetic material—collectively referred to as its **genome**—is organized inside of the cell. Since an organism's genetics to a large extent dictate its characteristics, it should not be surprising that organisms differ in the arrangement of their DNA and RNA.

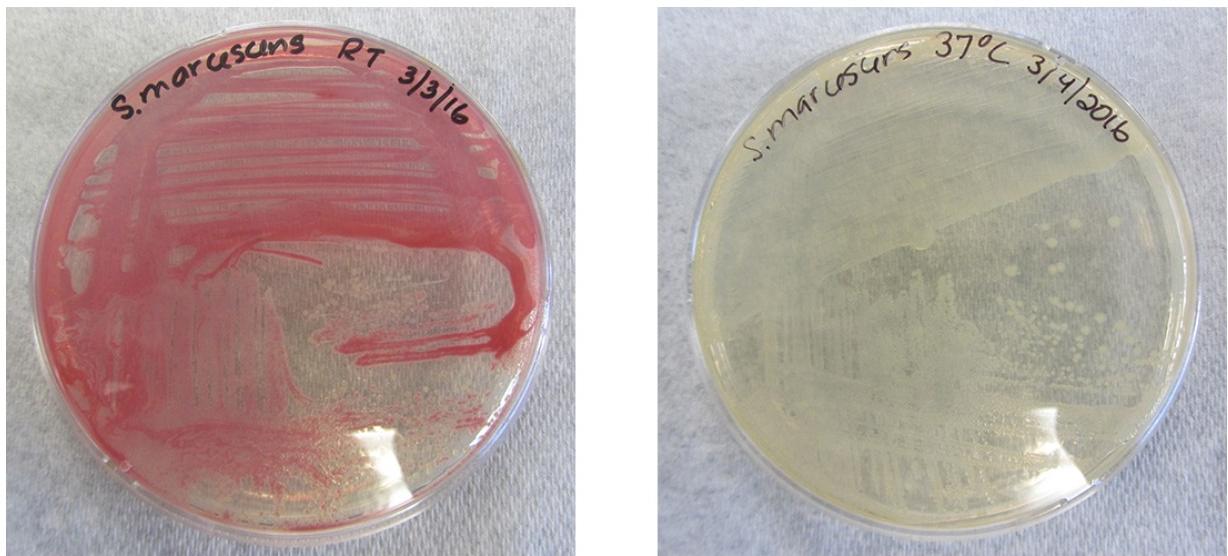
Genotype versus Phenotype

All cellular activities are encoded within a cell's DNA. The sequence of bases within a DNA molecule represents the genetic information of the cell. Segments of DNA molecules are called **genes**, and individual genes contain the instructional code necessary for synthesizing various proteins, enzymes, or stable RNA molecules.

The full collection of genes that a cell contains within its genome is called its **genotype**. However, a cell does not express all of its genes simultaneously. Instead, it turns on (expresses) or turns off certain genes when necessary. The set of genes being expressed at any given point in time

determines the cell's activities and its observable characteristics, referred to as its **phenotype**. Genes that are always expressed are known as constitutive genes; some constitutive genes are known as housekeeping genes because they are necessary for the basic functions of the cell.

While the genotype of a cell remains constant, the phenotype may change in response to environmental signals (e.g., changes in temperature or nutrient availability) that affect which nonconstitutive genes are expressed. For example, the oral bacterium *Streptococcus mutans* produces a sticky slime layer that allows it to adhere to teeth, forming dental plaque; however, the genes that control the production of the slime layer are only expressed in the presence of sucrose (table sugar). Thus, while the genotype of *S. mutans* is constant, its phenotype changes depending on the presence and absence of sugar in its environment. Temperature can also regulate gene expression. For example, the gram-negative bacterium *Serratia marcescens*, a pathogen frequently associated with hospital-acquired infections, produces a red pigment at 28 °C but not at 37 °C, the normal internal temperature of the human body ([\[link\]](#)).



Both plates contain strains of *Serratia marcescens* that have the gene for red pigment. However, this gene is expressed at 28 °C (left) but not at 37 °C (right). (credit: modification of work by Ann Auman)

Organization of Genetic Material

The vast majority of an organism's genome is organized into the cell's **chromosomes**, which are discrete DNA structures within cells that control cellular activity. Recall that while eukaryotic chromosomes are housed in the membrane-bound nucleus, most prokaryotes contain a single, circular chromosome that is found in an area of the cytoplasm called the nucleoid (see [Unique Characteristics of Prokaryotic Cells](#)). There is a big variation in genome size, and a chromosome may contain several thousand genes.

Organization of Eukaryotic Chromosome

Chromosome structure differs somewhat between eukaryotic and prokaryotic cells. Eukaryotic chromosomes are typically linear, and eukaryotic cells contain multiple distinct chromosomes. Many eukaryotic cells contain two copies of each chromosome and, therefore, are **diploid**.

The length of a chromosome greatly exceeds the length of the cell, so a chromosome needs to be packaged into a very small space to fit within the cell. For example, the combined length of all of the 3 billion base pairs[[footnote](#)] of DNA of the human genome would measure approximately 2 meters if completely stretched out, and some eukaryotic genomes are many times larger than the human genome. DNA **supercoiling** refers to the process by which DNA is twisted to fit inside the cell. Supercoiling may result in DNA that is either underwound (less than one turn of the helix per 10 base pairs) or overwound (more than one turn per 10 base pairs) from its normal relaxed state. Proteins known to be involved in supercoiling include **topoisomerases**; these enzymes help maintain the structure of supercoiled chromosomes, preventing overwinding of DNA during certain cellular processes like DNA replication.

National Human Genome Research Institute. "The Human Genome Project Completion: Frequently Asked Questions."

<https://www.genome.gov/11006943>. Accessed June 10, 2016

During **DNA packaging**, DNA-binding proteins called **histones** perform various levels of DNA wrapping and attachment to scaffolding proteins. The combination of DNA with these attached proteins is referred to as **chromatin**. In eukaryotes, the packaging of DNA by histones may be influenced by environmental factors that affect the presence of methyl groups on certain cytosine nucleotides of DNA. The influence of environmental factors on DNA packaging is called epigenetics. Epigenetics is another mechanism for regulating gene expression without altering the sequence of nucleotides. Epigenetic changes can be maintained through multiple rounds of cell division and, therefore, can be heritable.

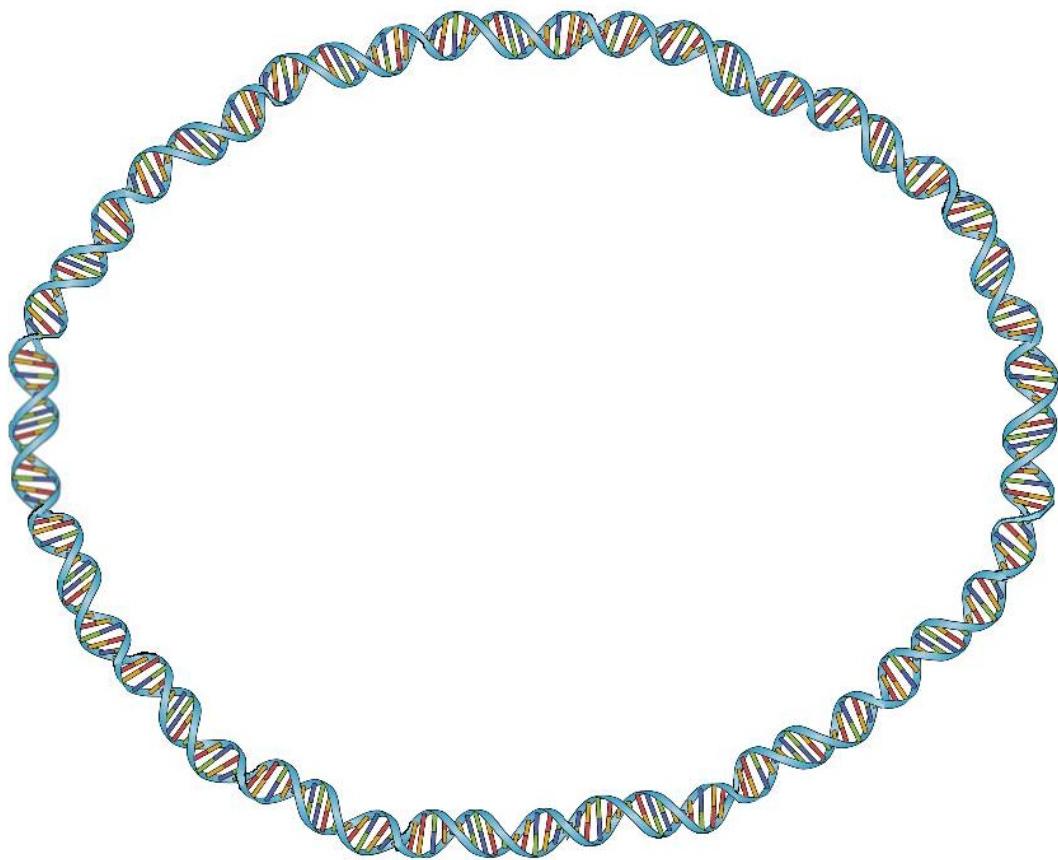
Note:



View this [animation](#) from the DNA Learning Center to learn more about on DNA packaging in eukaryotes.

Organization of Prokaryotic Chromosomes

Chromosomes in bacteria and archaea are usually **circular**, and a prokaryotic cell typically contains only a **single chromosome** within the nucleoid. Because the chromosome contains only one copy of each gene, prokaryotes are **haploid**.



Circular chromosome found in prokaryotes.

As in eukaryotic cells, DNA supercoiling is necessary for the genome to fit within the prokaryotic cell. The DNA in the bacterial chromosome is arranged in several supercoiled domains. As with eukaryotes, topoisomerases are involved in supercoiling DNA. DNA gyrase is a type of topoisomerase, found in bacteria and some archaea, that helps prevent the overwinding of DNA. (Some antibiotics kill bacteria by targeting DNA gyrase.) In addition, histone-like proteins bind DNA and aid in DNA packaging. Other proteins bind to the origin of replication, the location in the chromosome where DNA replication initiates. Because different regions of DNA are packaged differently, some regions of chromosomal DNA are more accessible to enzymes and thus may be used more readily as templates for gene expression. Interestingly, several bacteria, including *Helicobacter*

pylori and *Shigella flexneri*, have been shown to induce epigenetic changes in their hosts upon infection, leading to chromatin remodeling that may cause long-term effects on host immunity.[\[footnote\]](#)

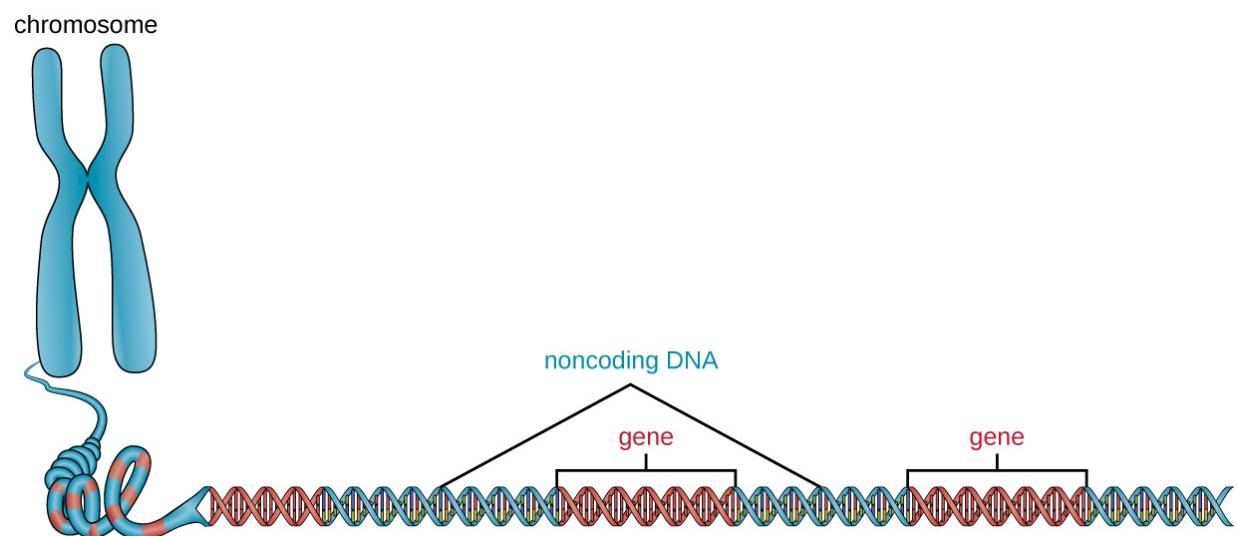
H. Bierne et al. “Epigenetics and Bacterial Infections.” *Cold Spring Harbor Perspectives in Medicine* 2 no. 12 (2012):a010272.

Note:

- What is the difference between a cell’s genotype and its phenotype?
- How does DNA fit inside cells?

Noncoding DNA

In addition to genes, a genome also contains many regions of **noncoding DNA** that do not encode proteins or stable RNA products. Noncoding DNA is commonly found in areas prior to the start of coding sequences of genes as well as in intergenic regions (i.e., DNA sequences located between genes) ([\[link\]](#)).



Chromosomes typically have a significant amount of noncoding DNA, often found in intergenic regions.

Prokaryotes appear to use their genomes very efficiently, with only an average of 12% of the genome being taken up by noncoding sequences. In contrast, noncoding DNA can represent about 98% of the genome in eukaryotes, as seen in humans, but the percentage of noncoding DNA varies between species.[\[footnote\]](#) These noncoding DNA regions were once referred to as “junk DNA”; however, this terminology is no longer widely accepted because scientists have since found roles for some of these regions, many of which contribute to the regulation of transcription or translation through the production of small noncoding RNA molecules, DNA packaging, and chromosomal stability. Although scientists may not fully understand the roles of all noncoding regions of DNA, it is generally believed that they do have purposes within the cell.

R.J. Taft et al. “The Relationship between Non-Protein-Coding DNA and Eukaryotic Complexity.” *Bioessays* 29 no. 3 (2007):288–299.

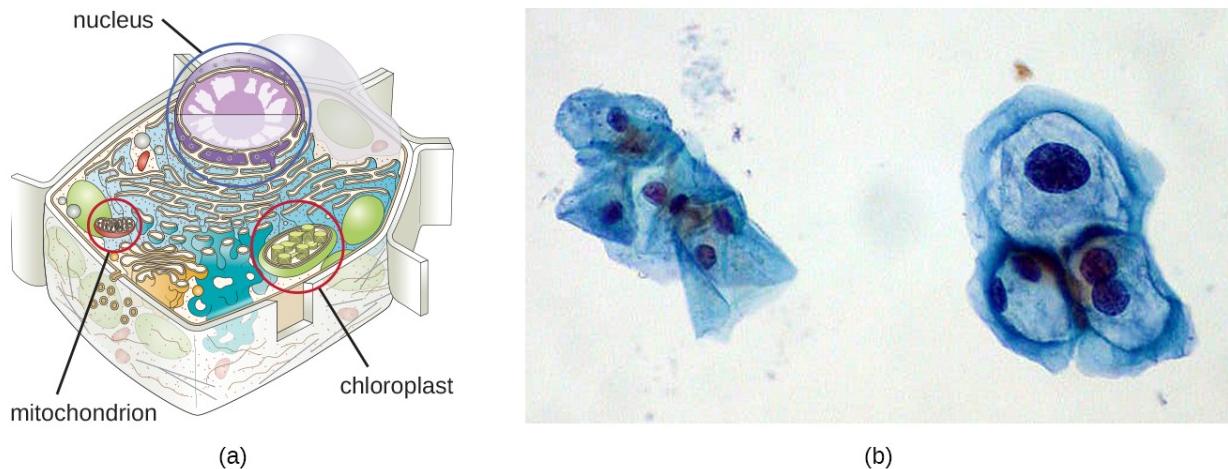
Note:

- What is the role of noncoding DNA?

Extrachromosomal DNA

Although most DNA is contained within a cell’s chromosomes, many cells have additional molecules of DNA outside the chromosomes, called **extrachromosomal DNA**, that are also part of its genome. The genomes of eukaryotic cells would also include the chromosomes from any organelles such as mitochondria and/or chloroplasts that these cells maintain ([\[link\]](#)). The maintenance of circular chromosomes in these organelles is a vestige of their prokaryotic origins and supports the endosymbiotic theory (see

Foundations of Modern Cell Theory). In some cases, genomes of certain DNA viruses can also be maintained independently in host cells during latent viral infection. In these cases, these viruses are another form of extrachromosomal DNA. For example, the human papillomavirus (HPV) may be maintained in infected cells in this way.



The genome of a eukaryotic cell consists of the chromosome housed in the nucleus, and extrachromosomal DNA found in the mitochondria (all cells) and chloroplasts (plants and algae).

Besides chromosomes, some prokaryotes also have smaller loops of DNA called plasmids that may contain one or a few genes not essential for normal growth ([\[link\]](#)). Bacteria can exchange these plasmids with other bacteria in a process known as horizontal gene transfer (HGT). The exchange of genetic material on plasmids sometimes provides microbes with new genes beneficial for growth and survival under special conditions. In some cases, genes obtained from plasmids may have clinical implications, encoding virulence factors that give a microbe the ability to cause disease or make a microbe resistant to certain antibiotics. Plasmids are also used heavily in genetic engineering and biotechnology as a way to move genes from one cell to another. The role of plasmids in horizontal

gene transfer and biotechnology will be discussed further in [Mechanisms of Microbial Genetics](#) and [Modern Applications of Microbial Genetics](#).

Note:

- How are plasmids involved in antibiotic resistance?

Viral Genomes

Viral genomes exhibit significant diversity in structure. Some viruses have genomes that consist of DNA as their genetic material. This DNA may be single stranded, as exemplified by human parvoviruses, or double stranded, as seen in the herpesviruses and poxviruses. Additionally, although all cellular life uses DNA as its genetic material, some viral genomes are made of either single-stranded or double-stranded RNA molecules, as we have discussed. Viral genomes are typically smaller than most bacterial genomes, encoding only a few genes, because they rely on their hosts to carry out many of the functions required for their replication. The diversity of viral genome structures and their implications for viral replication life cycles are discussed in more detail in [The Viral Life Cycle](#).

Note:

- Why do viral genomes vary widely among viruses?

Note:

Genome Size Matters

There is great variation in size of genomes among different organisms. Most eukaryotes maintain multiple chromosomes; humans, for example have 23 pairs, giving them 46 chromosomes. Despite being large at 3 billion base pairs, the human genome is far from the largest genome. Plants often maintain very large genomes, up to 150 billion base pairs, and commonly are polyploid, having multiple copies of each chromosome. The size of bacterial genomes also varies considerably, although they tend to be smaller than eukaryotic genomes ([\[link\]](#)). Some bacterial genomes may be as small as only 112,000 base pairs. Often, the size of a bacterium's genome directly relates to how much the bacterium depends on its host for survival. When a bacterium relies on the host cell to carry out certain functions, it loses the genes encoding the abilities to carry out those functions itself. These types of bacterial endosymbionts are reminiscent of the prokaryotic origins of mitochondria and chloroplasts.

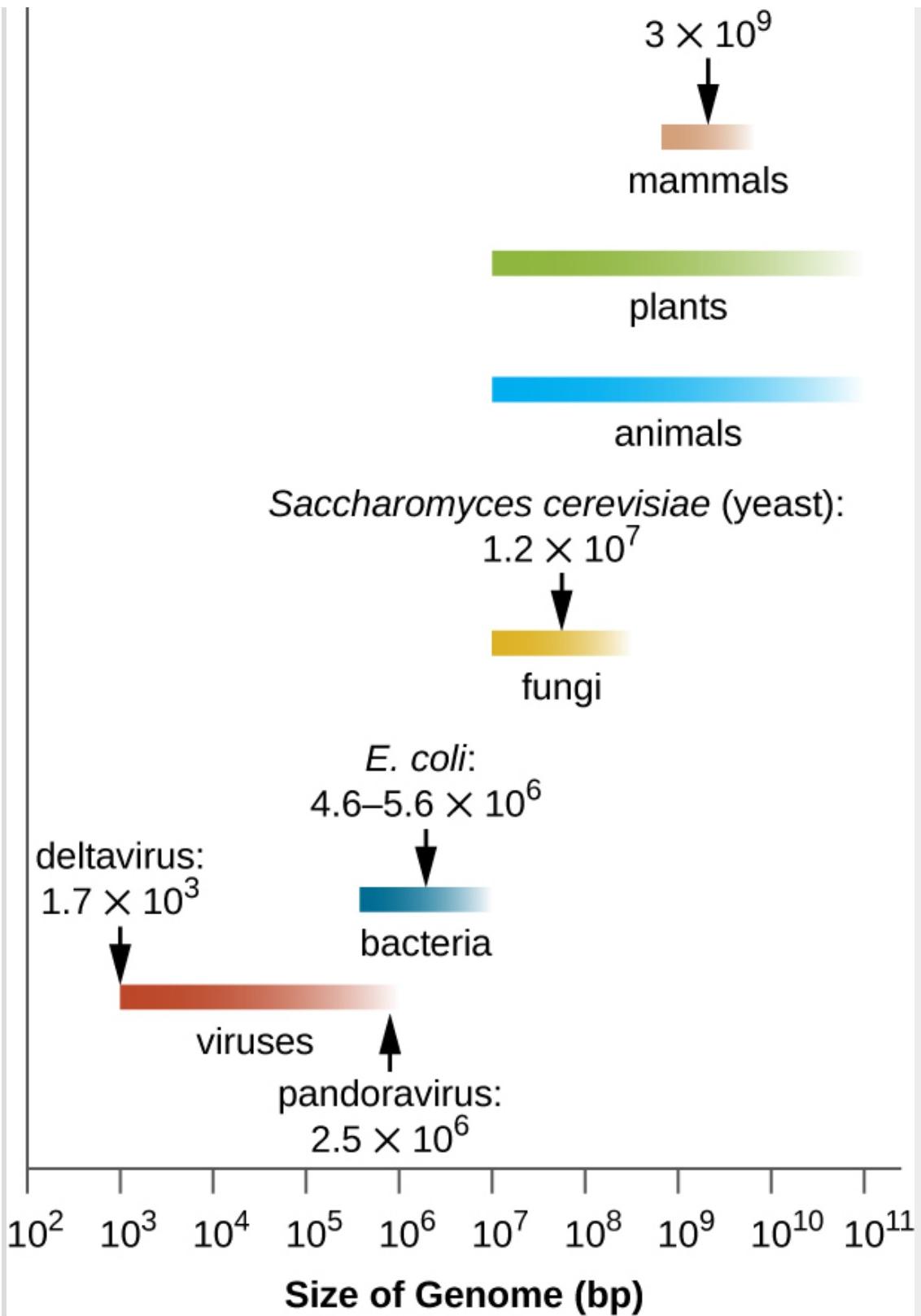
From a clinical perspective, obligate intracellular pathogens also tend to have small genomes (some around 1 million base pairs). Because host cells supply most of their nutrients, they tend to have a reduced number of genes encoding metabolic functions. Due to their small sizes, the genomes of organisms like *Mycoplasma genitalium* (580,000 base pairs), *Chlamydia trachomatis* (1.0 million), *Rickettsia prowazekii* (1.1 million), and *Treponema pallidum* (1.1 million) were some of the earlier bacterial genomes sequenced. Respectively, these pathogens cause urethritis and pelvic inflammation, chlamydia, typhus, and syphilis.

Whereas obligate intracellular pathogens have unusually small genomes, other bacteria with a great variety of metabolic and enzymatic capabilities have unusually large bacterial genomes. *Pseudomonas aeruginosa*, for example, is a bacterium commonly found in the environment and is able to grow on a wide range of substrates. Its genome contains 6.3 million base pairs, giving it a high metabolic ability and the ability to produce virulence factors that cause several types of opportunistic infections.

Interestingly, there has been significant variability in genome size in viruses as well, ranging from 3,500 base pairs to 2.5 million base pairs, significantly exceeding the size of many bacterial genomes. The great variation observed in viral genome sizes further contributes to the great diversity of viral genome characteristics already discussed.



humans:



There is great variability as well as overlap among the genome

sizes of various groups of organisms and viruses.

Note:



Visit the [genome database](#) of the National Center for Biotechnology Information (NCBI) to see the genomes that have been sequenced and their sizes.

Key Concepts and Summary

- The entire genetic content of a cell is its **genome**.
- **Genes** code for proteins, or stable RNA molecules, each of which carries out a specific function in the cell.
- Although the **genotype** that a cell possesses remains constant, expression of genes is dependent on environmental conditions.
- A **phenotype** is the observable characteristics of a cell (or organism) at a given point in time and results from the complement of genes currently being used.
- The majority of genetic material is organized into **chromosomes** that contain the DNA that controls cellular activities.
- Prokaryotes are typically haploid, usually having a single circular chromosome found in the nucleoid. Eukaryotes are diploid; DNA is organized into multiple linear chromosomes found in the nucleus.

- Supercoiling and DNA packaging using DNA binding proteins allows lengthy molecules to fit inside a cell. Eukaryotes and archaea use histone proteins, and bacteria use different proteins with similar function.
- Prokaryotic and eukaryotic genomes both contain **noncoding DNA**, the function of which is not well understood. Some noncoding DNA appears to participate in the formation of small noncoding RNA molecules that influence gene expression; some appears to play a role in maintaining chromosomal structure and in DNA packaging.
- **Extrachromosomal DNA** in eukaryotes includes the chromosomes found within organelles of prokaryotic origin (mitochondria and chloroplasts) that evolved by endosymbiosis. Some viruses may also maintain themselves extrachromosomally.
- Extrachromosomal DNA in prokaryotes is commonly maintained as **plasmids** that encode a few nonessential genes that may be helpful under specific conditions. Plasmids can be spread through a bacterial community by horizontal gene transfer.
- Viral genomes show extensive variation and may be composed of either RNA or DNA, and may be either double or single stranded.

Critical Thinking

Exercise:

Problem:

A new type of bacteriophage has been isolated and you are in charge of characterizing its genome. The base composition of the bacteriophage is A (15%), C (20%), T (35%), and G (30%). What can you conclude about the genome of the virus?

Mechanisms of Microbial Genetics - Introduction

class="introduction"

Escherichia

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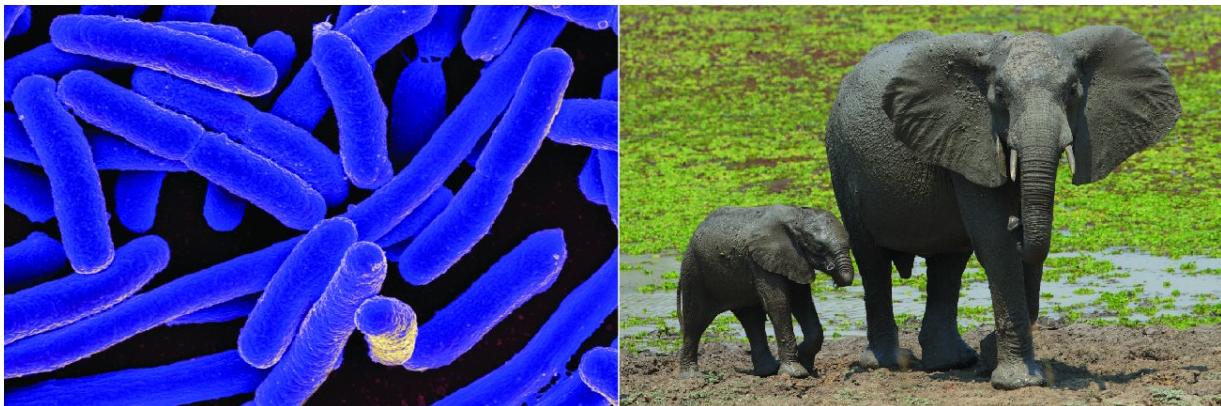
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In 1954, French scientist and future Nobel laureate Jacques Monod (1910–1976) famously said, “What is true in *E. coli* is true in the elephant,” suggesting that the biochemistry of life was maintained throughout evolution and is shared in all forms of known life. Since Monod’s famous statement, we have learned a great deal about the mechanisms of gene regulation, expression, and replication in living cells. All cells use DNA for information storage, share the same genetic code, and use similar mechanisms to replicate and express it. Although many aspects of genetics are universally shared, variations do exist among contemporary genetic systems. We now know that within the shared overall theme of the genetic mechanism, there are significant differences among the three domains of life: Eukarya, Archaea, and Bacteria. Additionally, viruses, cellular parasites but not themselves living cells, show dramatic variation in their genetic material and the replication and gene expression processes. Some of these differences have allowed us to engineer clinical tools such as antibiotics and antiviral drugs that specifically inhibit the reproduction of pathogens yet are harmless to their hosts.

The Functions of Genetic Material

LEARNING OBJECTIVES

- Explain the two functions of the genome
- Explain the meaning of the central dogma of molecular biology
- Differentiate between genotype and phenotype and explain how environmental factors influence phenotype

DNA serves two essential functions that deal with cellular information. First, DNA is the genetic material responsible for inheritance and is passed from parent to offspring for all life on earth. To preserve the integrity of this genetic information, DNA must be replicated with great accuracy, with minimal errors that introduce changes to the DNA sequence. A genome contains the full complement of DNA within a cell and is organized into smaller, discrete units called genes that are arranged on chromosomes and plasmids. The second function of DNA is to direct and regulate the construction of the proteins necessary to a cell for growth and reproduction in a particular cellular environment.

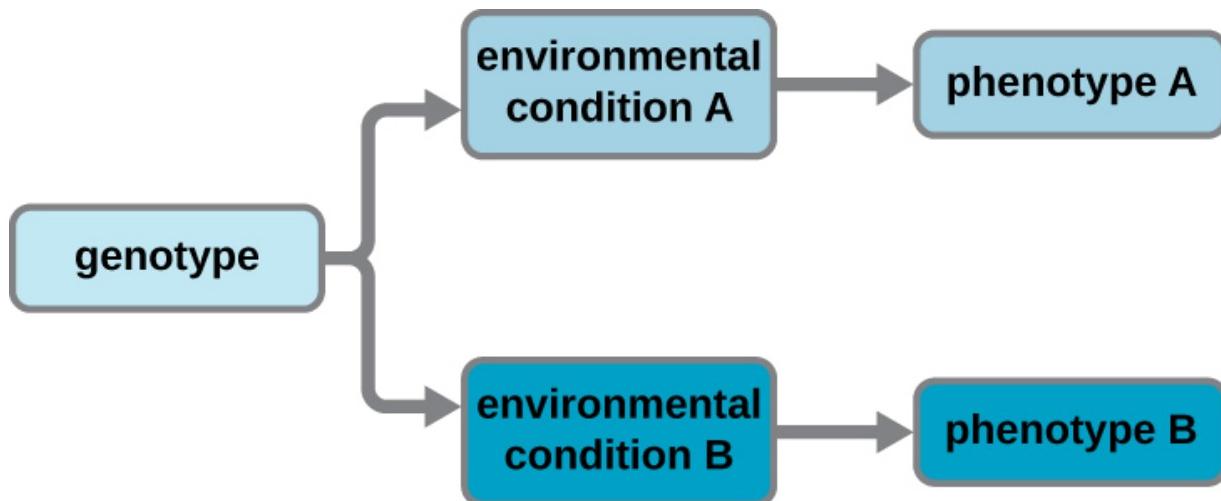
A gene is composed of DNA that is “read” or transcribed to produce an RNA molecule during the process of transcription. One major type of RNA molecule, called messenger RNA (mRNA), provides the information for the ribosome to catalyze protein synthesis in a process called translation. The processes of transcription and translation are collectively referred to as **gene expression**. Gene expression is the synthesis of a specific protein with a sequence of amino acids that is encoded in the gene. The flow of genetic information from DNA to RNA to protein is described by the **central**

dogma ([\[link\]](#)). This central dogma of molecular biology further elucidates the mechanism behind Beadle and Tatum’s “one gene-one enzyme” hypothesis (see [Using Microorganisms to Discover the Secrets of Life](#)). Each of the processes of replication, transcription, and translation includes the stages of 1) initiation, 2) elongation (polymerization), and 3) termination. These stages will be described in more detail in this chapter.



The central dogma states that DNA encodes messenger RNA, which, in turn, encodes protein.

A cell’s genotype is the full collection of genes it contains, whereas its phenotype is the set of observable characteristics that result from those genes. The phenotype is the product of the array of proteins being produced by the cell at a given time, which is influenced by the cell’s genotype as well as interactions with the cell’s environment. Genes code for proteins that have functions in the cell. Production of a specific protein encoded by an individual gene often results in a distinct phenotype for the cell compared with the phenotype without that protein. For this reason, it is also common to refer to the genotype of an individual gene and its phenotype. Although a cell’s genotype remains constant, not all genes are used to direct the production of their proteins simultaneously. Cells carefully regulate expression of their genes, only using genes to make specific proteins when those proteins are needed ([\[link\]](#)).



Phenotype is determined by the specific genes within a genotype that are expressed under specific conditions. Although multiple cells may have the same genotype, they may exhibit a wide range of phenotypes resulting from differences in patterns of gene expression in response to different environmental conditions.

Note:

- What are the two functions of DNA?
- Distinguish between the genotype and phenotype of a cell.
- How can cells have the same genotype but differ in their phenotype?

Note:

Use and Abuse of Genome Data

Why can some humans harbor opportunistic pathogens like *Haemophilus influenzae*, *Staphylococcus aureus*, or *Streptococcus pyogenes*, in their upper respiratory tracts but remain asymptomatic carriers, while other individuals become seriously ill when infected? There is evidence suggesting that differences in susceptibility to infection between patients

may be a result, at least in part, of genetic differences between human hosts. For example, genetic differences in human leukocyte antigens (HLAs) and red blood cell antigens among hosts have been implicated in different immune responses and resulting disease progression from infection with *H. influenzae*.

Because the genetic interplay between pathogen and host may contribute to disease outcomes, understanding differences in genetic makeup between individuals may be an important clinical tool. Ecological genomics is a relatively new field that seeks to understand how the genotypes of different organisms interact with each other in nature. The field answers questions about how gene expression of one organism affects gene expression of another. Medical applications of ecological genomics will focus on how pathogens interact with specific individuals, as opposed to humans in general. Such analyses would allow medical professionals to use knowledge of an individual's genotype to apply more individualized plans for treatment and prevention of disease.

With the advent of next-generation sequencing, it is relatively easy to obtain the entire genomic sequences of pathogens; a bacterial genome can be sequenced in as little as a day.[\[footnote\]](#) The speed and cost of sequencing the human genome has also been greatly reduced and, already, individuals can submit samples to receive extensive reports on their personal genetic traits, including ancestry and carrier status for various genetic diseases. As sequencing technologies progress further, such services will continue to become less expensive, more extensive, and quicker.

D.J. Edwards, K.E. Holt. "Beginner's Guide to Comparative Bacterial Genome Analysis Using Next-Generation Sequence Data." *Microbial Informatics and Experimentation* 3 no. 1 (2013):2.

However, as this day quickly approaches, there are many ethical concerns with which society must grapple. For example, should genome sequencing be a standard practice for everybody? Should it be required by law or by employers if it will lower health-care costs? If one refuses genome sequencing, does he or she forfeit his or her right to health insurance coverage? For what purposes should the data be used? Who should oversee proper use of these data? If genome sequencing reveals predisposition to a particular disease, do insurance companies have the right to increase rates? Will employers treat an employee differently? Knowing that environmental

influences also affect disease development, how should the data on the presence of a particular disease-causing allele in an individual be used ethically? The Genetic Information Nondiscrimination Act of 2008 (GINA) currently prohibits discriminatory practices based on genetic information by both health insurance companies and employers. However, GINA does not cover life, disability, or long-term care insurance policies. Clearly, all members of society must continue to engage in conversations about these issues so that such genomic data can be used to improve health care while simultaneously protecting an individual's rights.

Key Concepts and Summary

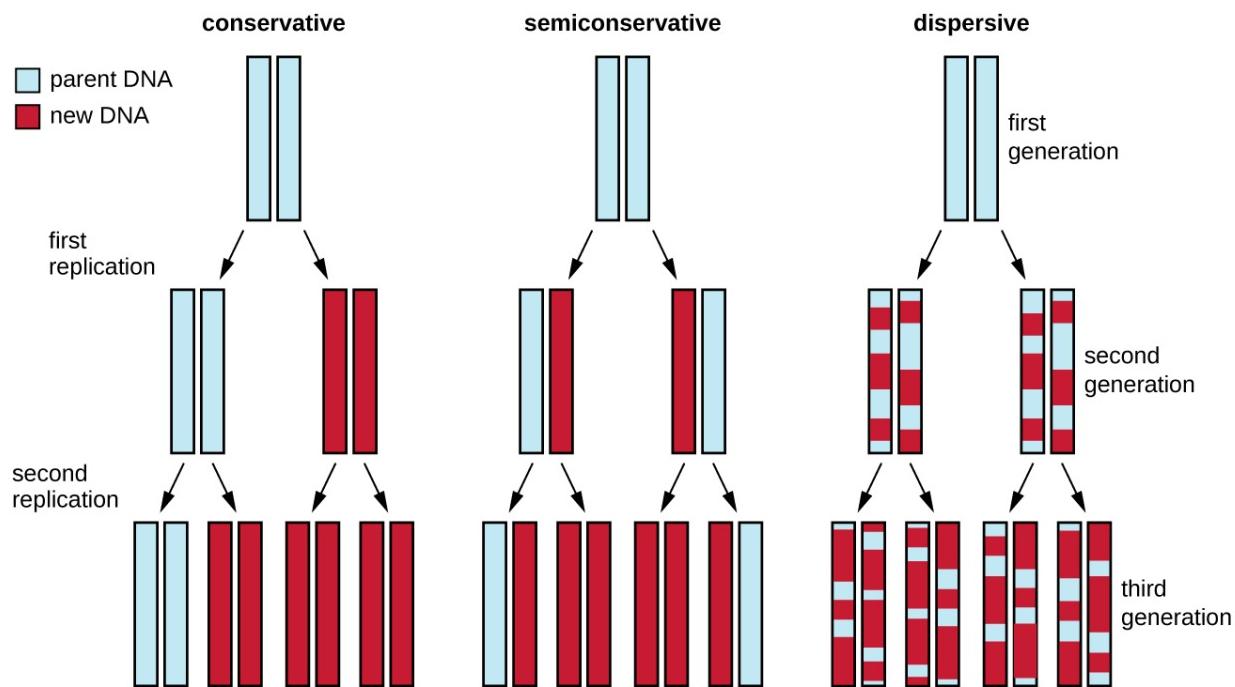
- DNA serves two important cellular functions: It is the genetic material passed from parent to offspring and it serves as the information to direct and regulate the construction of the proteins necessary for the cell to perform all of its functions.
- The **central dogma** states that DNA organized into genes specifies the sequences of messenger RNA (mRNA), which, in turn, specifies the amino acid sequence of proteins.
- The genotype of a cell is the full collection of genes a cell contains. Not all genes are used to make proteins simultaneously. The phenotype is a cell's observable characteristics resulting from the proteins it is producing at a given time under specific environmental conditions.

DNA Replication

LEARNING OBJECTIVES

- Explain the meaning of semiconservative DNA replication
- Explain why DNA replication is bidirectional and includes both a leading and lagging strand
- Explain why Okazaki fragments are formed
- Describe the process of DNA replication and the functions of the enzymes involved
- Identify the differences between DNA replication in bacteria and eukaryotes
- Explain the process of rolling circle replication

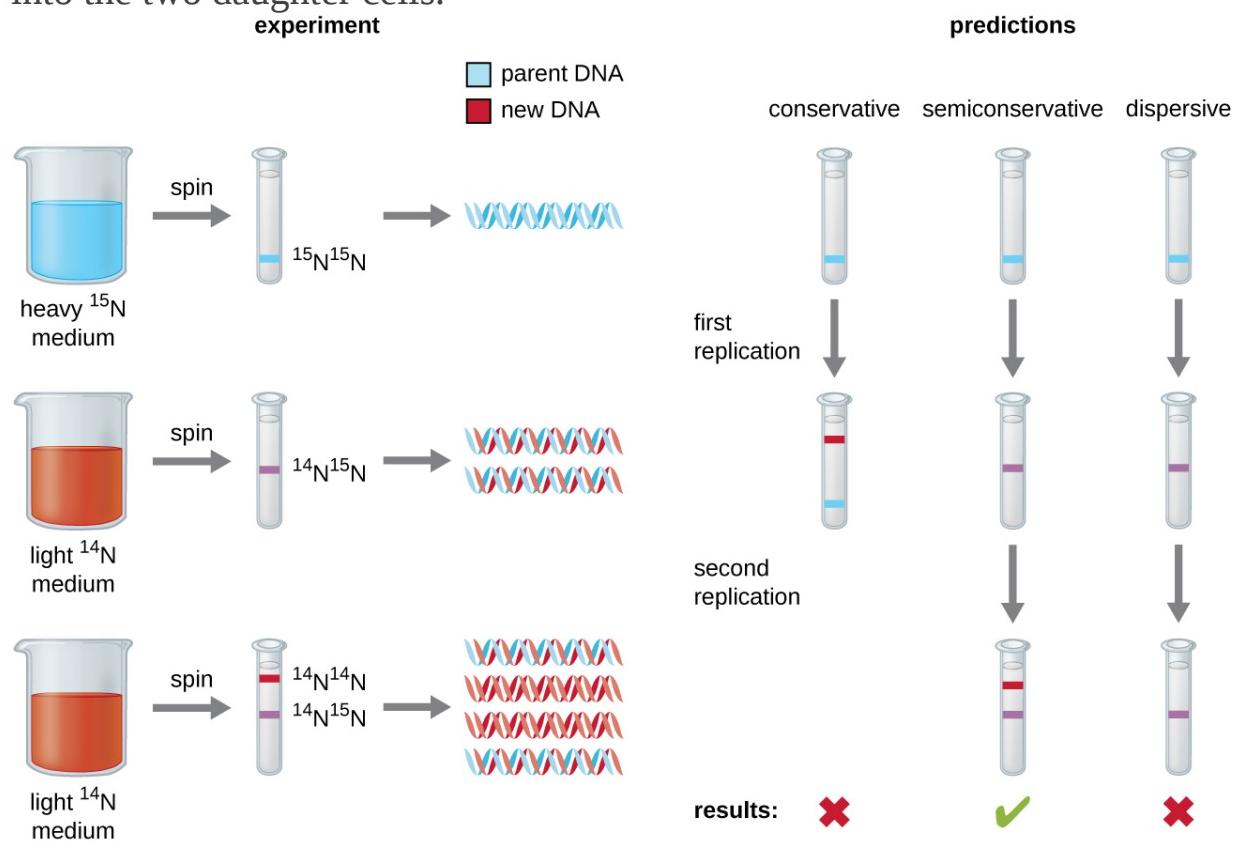
The elucidation of the structure of the double helix by James Watson and Francis Crick in 1953 provided a hint as to how DNA is copied during the process of **replication**. Separating the strands of the double helix would provide two templates for the synthesis of new complementary strands, but exactly how new DNA molecules were constructed was still unclear. In one model, **semiconservative replication**, the two strands of the double helix separate during DNA replication, and each strand serves as a template from which the new complementary strand is copied; after replication, each double-stranded DNA includes one parental or “old” strand and one “new” strand. There were two competing models also suggested: conservative and dispersive, which are shown in [\[link\]](#).



There were three models suggested for DNA replication. In the conservative model, parental DNA strands (blue) remained associated in one DNA molecule while new daughter strands (red) remained associated in newly formed DNA molecules. In the semiconservative model, parental strands separated and directed the synthesis of a daughter strand, with each resulting DNA molecule being a hybrid of a parental strand and a daughter strand. In the dispersive model, all resulting DNA strands have regions of double-stranded parental DNA and regions of double-stranded daughter DNA.

Matthew Meselson (1930–) and Franklin Stahl (1929–) devised an experiment in 1958 to test which of these models correctly represents DNA replication ([\[link\]](#)). They grew *E. coli* for several generations in a medium containing a “heavy” isotope of nitrogen (^{15}N) that was incorporated into nitrogenous bases and, eventually, into the DNA. This labeled the parental DNA. The *E. coli* culture was then shifted into a medium containing ^{14}N and allowed to grow for one generation. The cells were harvested and the DNA was isolated. The DNA was separated by ultracentrifugation, during which the DNA formed bands according to its density. DNA grown in ^{15}N

would be expected to form a band at a higher density position than that grown in ^{14}N . Meselson and Stahl noted that after one generation of growth in ^{14}N , the single band observed was intermediate in position in between DNA of cells grown exclusively in ^{15}N or ^{14}N . This suggested either a semiconservative or dispersive mode of replication. Some cells were allowed to grow for one more generation in ^{14}N and spun again. The DNA harvested from cells grown for two generations in ^{14}N formed two bands: one DNA band was at the intermediate position between ^{15}N and ^{14}N , and the other corresponded to the band of ^{14}N DNA. These results could only be explained if DNA replicates in a semiconservative manner. Therefore, the other two models were ruled out. As a result of this experiment, we now know that during DNA replication, each of the two strands that make up the double helix serves as a template from which new strands are copied. The new strand will be complementary to the parental or “old” strand. The resulting DNA molecules have the same sequence and are divided equally into the two daughter cells.



Meselson and Stahl experimented with *E. coli* grown first in heavy nitrogen (^{15}N) then in ^{14}N . DNA grown in ^{15}N (blue band) was heavier

than DNA grown in ^{14}N (red band), and sedimented to a lower level on ultracentrifugation. After one round of replication, the DNA sedimented halfway between the ^{15}N and ^{14}N levels (purple band), ruling out the conservative model of replication. After a second round of replication, the dispersive model of replication was ruled out. These data supported the semiconservative replication model.

Note:

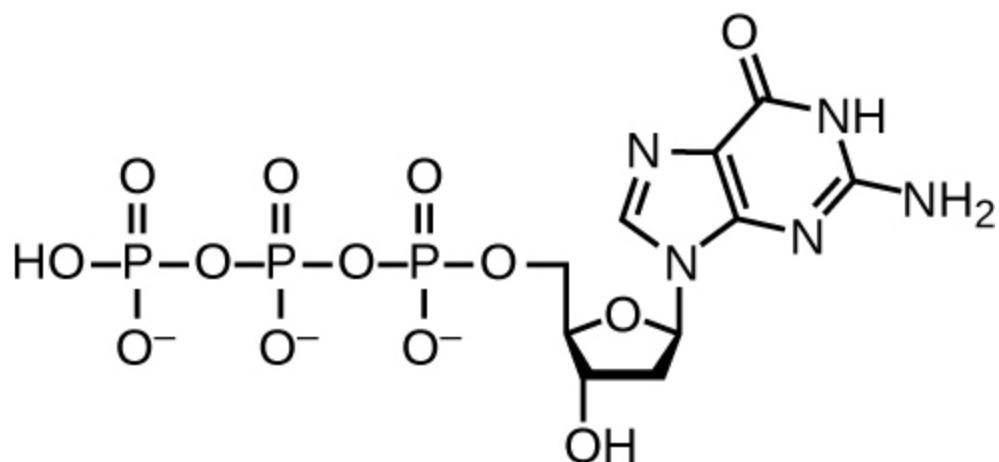
- What would have been the conclusion of Meselson and Stahl's experiment if, after the first generation, they had found two bands of DNA?

DNA Replication in Bacteria

DNA replication has been well studied in bacteria primarily because of the small size of the genome and the mutants that are available. *E. coli* has 4.6 million base pairs (Mbp) in a single circular chromosome and all of it is replicated in approximately 42 minutes, starting from a single origin of replication and proceeding around the circle bidirectionally (i.e., in both directions). This means that approximately 1000 nucleotides are added per second. The process is quite rapid and occurs with few errors.

DNA replication uses a large number of proteins and enzymes ([\[link\]](#)). One of the key players is the enzyme **DNA polymerase**, also known as DNA pol. In bacteria, three main types of DNA polymerases are known: DNA pol I, DNA pol II, and DNA pol III. It is now known that DNA pol III is the enzyme required for DNA synthesis; DNA pol I and DNA pol II are primarily required for repair. DNA pol III adds deoxyribonucleotides each complementary to a nucleotide on the template strand, one by one to the 3'-OH group of the growing DNA chain. The addition of these nucleotides

requires energy. This energy is present in the bonds of three phosphate groups attached to each nucleotide (a triphosphate nucleotide), similar to how energy is stored in the phosphate bonds of adenosine triphosphate (ATP) ([\[link\]](#)). When the bond between the phosphates is broken and diphosphate is released, the energy released allows for the formation of a covalent phosphodiester bond by dehydration synthesis between the incoming nucleotide and the free 3'-OH group on the growing DNA strand.



This structure shows the guanosine triphosphate deoxyribonucleotide that is incorporated into a growing DNA strand by cleaving the two end phosphate groups from the molecule and transferring the energy to the sugar phosphate bond. The other three nucleotides form analogous structures.

Initiation

The **initiation of replication** occurs at specific nucleotide sequence called the **origin of replication**, where various proteins bind to begin the replication process. *E. coli* has a single origin of replication (as do most

prokaryotes), called *oriC*, on its one chromosome. The origin of replication is approximately 245 base pairs long and is rich in adenine-thymine (AT) sequences.

Some of the proteins that bind to the origin of replication are important in making single-stranded regions of DNA accessible for replication.

Chromosomal DNA is typically wrapped around histones (in eukaryotes and archaea) or histone-like proteins (in bacteria), and is **supercoiled**, or extensively wrapped and twisted on itself. This packaging makes the information in the DNA molecule inaccessible. However, enzymes called topoisomerases change the shape and supercoiling of the chromosome. For bacterial DNA replication to begin, the supercoiled chromosome is relaxed by **topoisomerase II**, also called **DNA gyrase**. An enzyme called **helicase** then separates the DNA strands by breaking the hydrogen bonds between the nitrogenous base pairs. Recall that AT sequences have fewer hydrogen bonds and, hence, have weaker interactions than guanine-cytosine (GC) sequences. These enzymes require ATP hydrolysis. As the DNA opens up, Y-shaped structures called **replication forks** are formed. Two replication forks are formed at the origin of replication, allowing for bidirectional replication and formation of a structure that looks like a bubble when viewed with a transmission electron microscope; as a result, this structure is called a **replication bubble**. The DNA near each replication fork is coated with **single-stranded binding proteins** to prevent the single-stranded DNA from rewinding into a double helix.

Once single-stranded DNA is accessible at the origin of replication, DNA replication can begin. However, DNA pol III is able to add nucleotides only in the 5' to 3' direction (a new DNA strand can be only extended in this direction). This is because DNA polymerase requires a free 3'-OH group to which it can add nucleotides by forming a covalent phosphodiester bond between the 3'-OH end and the 5' phosphate of the next nucleotide. This also means that it cannot add nucleotides if a free 3'-OH group is not available, which is the case for a single strand of DNA. The problem is solved with the help of an RNA sequence that provides the free 3'-OH end. Because this sequence allows the start of DNA synthesis, it is appropriately called the **primer**. The primer is five to 10 nucleotides long and complementary to the parental or template DNA. It is synthesized by RNA

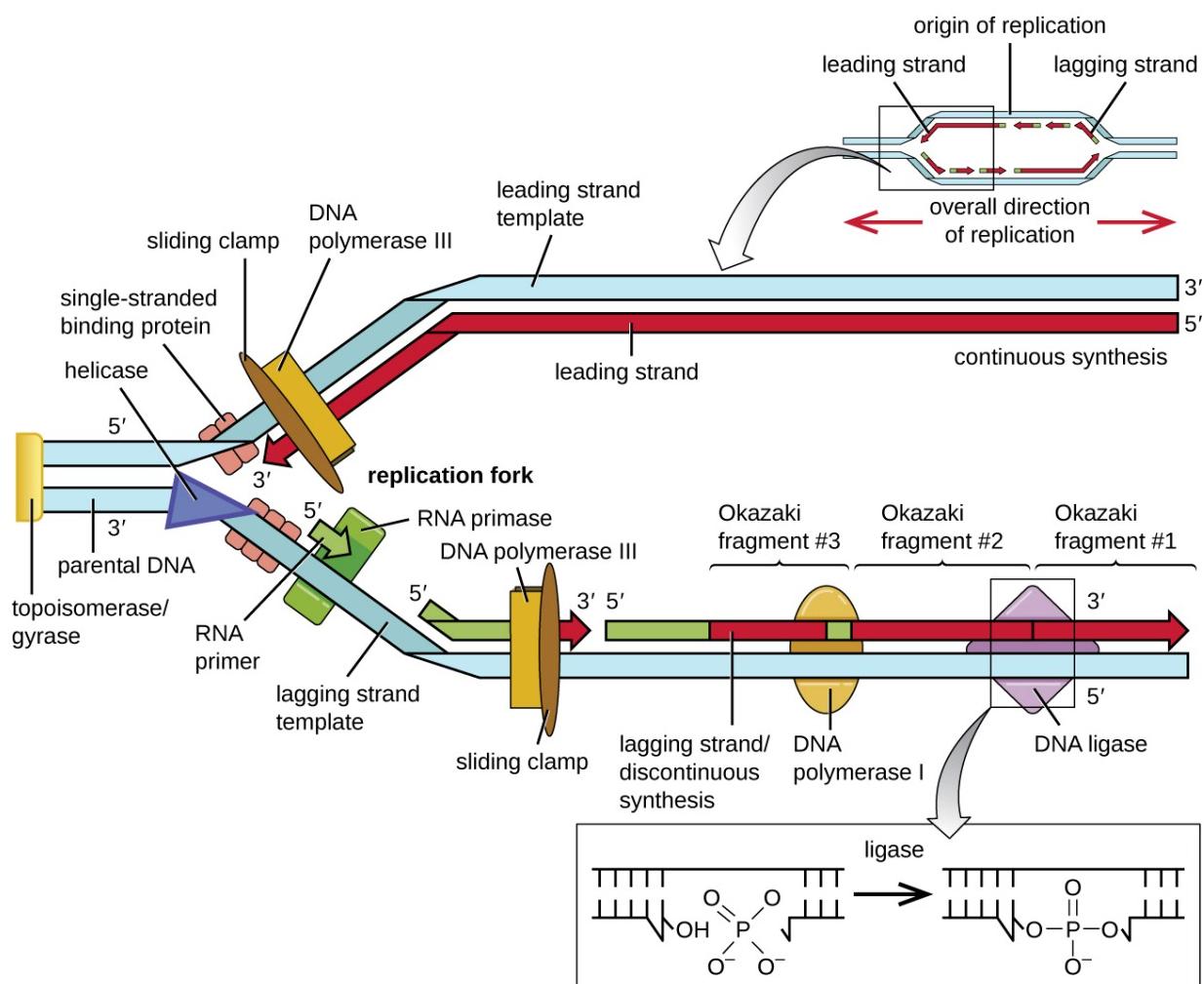
primase, which is an RNA polymerase. Unlike DNA polymerases, RNA polymerases do not need a free 3'-OH group to synthesize an RNA molecule. Now that the primer provides the free 3'-OH group, DNA polymerase III can now extend this RNA primer, adding DNA nucleotides one by one that are complementary to the template strand ([\[link\]](#)).

Elongation

During **elongation in DNA replication**, the addition of nucleotides occurs at its maximal rate of about 1000 nucleotides per second. DNA polymerase III can only extend in the 5' to 3' direction, which poses a problem at the replication fork. The DNA double helix is antiparallel; that is, one strand is oriented in the 5' to 3' direction and the other is oriented in the 3' to 5' direction (see [Structure and Function of DNA](#)). During replication, one strand, which is complementary to the 3' to 5' parental DNA strand, is synthesized continuously toward the replication fork because polymerase can add nucleotides in this direction. This continuously synthesized strand is known as the **leading strand**. The other strand, complementary to the 5' to 3' parental DNA, grows away from the replication fork, so the polymerase must move back toward the replication fork to begin adding bases to a new primer, again in the direction away from the replication fork. It does so until it bumps into the previously synthesized strand and then it moves back again ([\[link\]](#)). These steps produce small DNA sequence fragments known as **Okazaki fragments**, each separated by RNA primer. Okazaki fragments are named after the Japanese research team and married couple Reiji and Tsuneko Okazaki, who first discovered them in 1966. The strand with the Okazaki fragments is known as the **lagging strand**, and its synthesis is said to be discontinuous.

The leading strand can be extended from one primer alone, whereas the lagging strand needs a new primer for each of the short Okazaki fragments. The overall direction of the lagging strand will be 3' to 5', and that of the leading strand 5' to 3'. A protein called the sliding clamp holds the DNA polymerase in place as it continues to add nucleotides. The sliding clamp is a ring-shaped protein that binds to the DNA and holds the polymerase in place. Beyond its role in initiation, topoisomerase also prevents the

overwinding of the DNA double helix ahead of the replication fork as the DNA is opening up; it does so by causing temporary nicks in the DNA helix and then resealing it. As synthesis proceeds, the RNA primers are replaced by DNA. The primers are removed by the **exonuclease** activity of DNA polymerase I, and the gaps are filled in. The nicks that remain between the newly synthesized DNA (that replaced the RNA primer) and the previously synthesized DNA are sealed by the enzyme **DNA ligase** that catalyzes the formation of covalent phosphodiester linkage between the 3'-OH end of one DNA fragment and the 5' phosphate end of the other fragment, stabilizing the sugar-phosphate backbone of the DNA molecule.



At the origin of replication, topoisomerase II relaxes the supercoiled chromosome. Two replication forks are formed by the opening of the

double-stranded DNA at the origin, and helicase separates the DNA strands, which are coated by single-stranded binding proteins to keep the strands separated. DNA replication occurs in both directions. An RNA primer complementary to the parental strand is synthesized by RNA primase and is elongated by DNA polymerase III through the addition of nucleotides to the 3'-OH end. On the leading strand, DNA is synthesized continuously, whereas on the lagging strand, DNA is synthesized in short stretches called Okazaki fragments. RNA primers within the lagging strand are removed by the exonuclease activity of DNA polymerase I, and the Okazaki fragments are joined by DNA ligase.

Termination

Once the complete chromosome has been replicated, **termination of DNA replication** must occur. Although much is known about initiation of replication, less is known about the termination process. Following replication, the resulting complete circular genomes of prokaryotes are concatenated, meaning that the circular DNA chromosomes are interlocked and must be separated from each other. This is accomplished through the activity of bacterial topoisomerase IV, which introduces double-stranded breaks into DNA molecules, allowing them to separate from each other; the enzyme then reseals the circular chromosomes. The resolution of concatemers is an issue unique to prokaryotic DNA replication because of their circular chromosomes. Because both bacterial DNA gyrase and topoisomerase IV are distinct from their eukaryotic counterparts, these enzymes serve as targets for a class of antimicrobial drugs called quinolones.

The Molecular Machinery Involved in Bacterial DNA Replication

Enzyme or Factor	Function
DNA pol I	Exonuclease activity removes RNA primer and replaces it with newly synthesized DNA
DNA pol III	Main enzyme that adds nucleotides in the 5' to 3' direction
Helicase	Opens the DNA helix by breaking hydrogen bonds between the nitrogenous bases
Ligase	Seals the gaps between the Okazaki fragments on the lagging strand to create one continuous DNA strand
Primase	Synthesizes RNA primers needed to start replication
Single-stranded binding proteins	Bind to single-stranded DNA to prevent hydrogen bonding between DNA strands, reforming double-stranded DNA
Sliding clamp	Helps hold DNA pol III in place when nucleotides are being added
Topoisomerase II (DNA gyrase)	Relaxes supercoiled chromosome to make DNA more accessible for the initiation of replication; helps relieve the stress on DNA when unwinding, by causing breaks and then resealing the DNA

The Molecular Machinery Involved in Bacterial DNA Replication

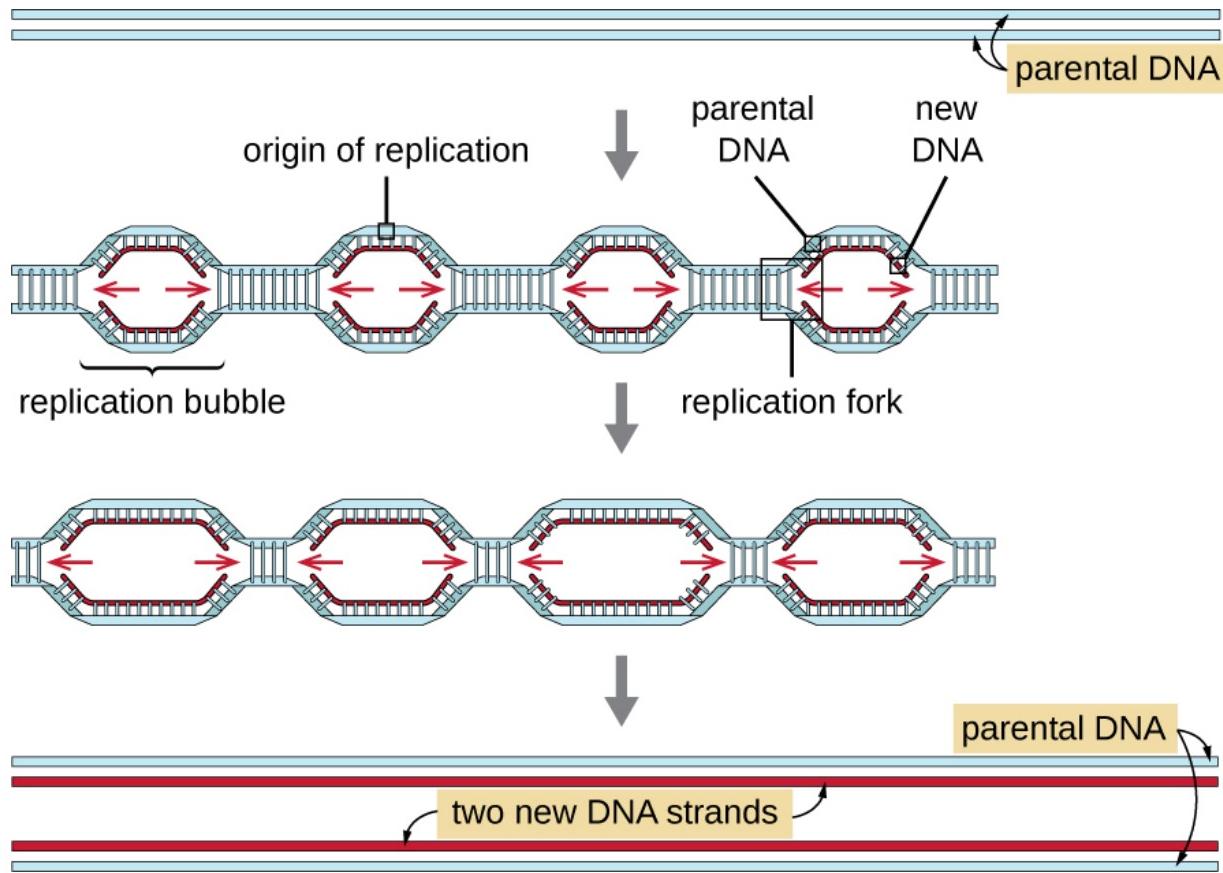
Enzyme or Factor	Function
Topoisomerase IV	Introduces single-stranded break into concatenated chromosomes to release them from each other, and then reseals the DNA

Note:

- Which enzyme breaks the hydrogen bonds holding the two strands of DNA together so that replication can occur?
- Is it the lagging strand or the leading strand that is synthesized in the direction toward the opening of the replication fork?
- Which enzyme is responsible for removing the RNA primers in newly replicated bacterial DNA?

DNA Replication in Eukaryotes

Eukaryotic genomes are much more complex and larger than prokaryotic genomes and are typically composed of multiple linear chromosomes ([\[link\]](#)). The human genome, for example, has 3 billion base pairs per haploid set of chromosomes, and 6 billion base pairs are inserted during replication. There are multiple origins of replication on each eukaryotic chromosome ([\[link\]](#)); the human genome has 30,000 to 50,000 origins of replication. The rate of replication is approximately 100 nucleotides per second—10 times slower than prokaryotic replication.



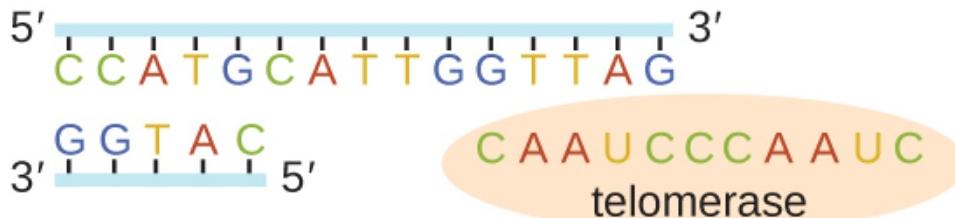
Eukaryotic chromosomes are typically linear, and each contains multiple origins of replication.

The essential steps of replication in eukaryotes are the same as in prokaryotes. Before replication can start, the DNA has to be made available as a template. Eukaryotic DNA is highly supercoiled and packaged, which is facilitated by many proteins, including histones (see [Structure and Function of Cellular Genomes](#)). At the origin of replication, a prereplication complex composed of several proteins, including helicase, forms and recruits other enzymes involved in the initiation of replication, including topoisomerase to relax supercoiling, single-stranded binding protein, RNA primase, and DNA polymerase. Following initiation of replication, in a process similar to that found in prokaryotes, elongation is facilitated by eukaryotic DNA polymerases. The leading strand is continuously synthesized by the eukaryotic polymerase enzyme pol δ , while the lagging strand

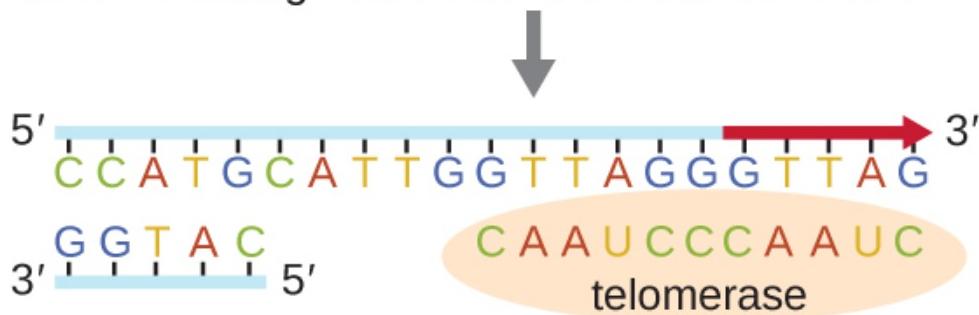
strand is synthesized by pol ε. A sliding clamp protein holds the DNA polymerase in place so that it does not fall off the DNA. The enzyme ribonuclease H (RNase H), instead of a DNA polymerase as in bacteria, removes the RNA primer, which is then replaced with DNA nucleotides. The gaps that remain are sealed by DNA ligase.

Because eukaryotic chromosomes are linear, one might expect that their replication would be more straightforward. As in prokaryotes, the eukaryotic DNA polymerase can add nucleotides only in the 5' to 3' direction. In the leading strand, synthesis continues until it reaches either the end of the chromosome or another replication fork progressing in the opposite direction. On the lagging strand, DNA is synthesized in short stretches, each of which is initiated by a separate primer. When the replication fork reaches the end of the linear chromosome, there is no place to make a primer for the DNA fragment to be copied at the end of the chromosome. These ends thus remain unpaired and, over time, they may get progressively shorter as cells continue to divide.

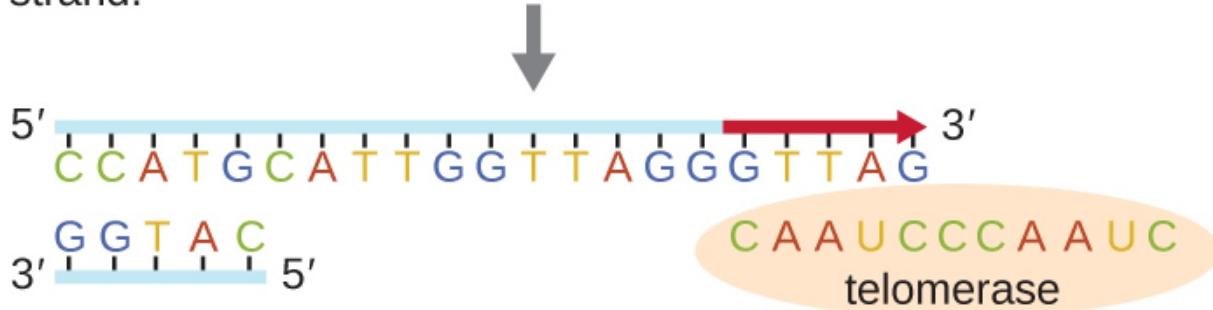
The ends of the linear chromosomes are known as **telomeres** and consist of noncoding repetitive sequences. The telomeres protect coding sequences from being lost as cells continue to divide. In humans, a six base-pair sequence, TTAGGG, is repeated 100 to 1000 times to form the telomere. The discovery of the enzyme **telomerase** ([\[link\]](#)) clarified our understanding of how chromosome ends are maintained. Telomerase contains a catalytic part and a built-in RNA template. It attaches to the end of the chromosome, and complementary bases to the RNA template are added on the 3' end of the DNA strand. Once the 3' end of the lagging strand template is sufficiently elongated, DNA polymerase can add the nucleotides complementary to the ends of the chromosomes. In this way, the ends of the chromosomes are replicated. In humans, telomerase is typically active in germ cells and adult stem cells; it is not active in adult somatic cells and may be associated with the aging of these cells. Eukaryotic microbes including fungi and protozoans also produce telomerase to maintain chromosomal integrity. For her discovery of telomerase and its action, Elizabeth Blackburn (1948–) received the Nobel Prize for Medicine or Physiology in 2009.



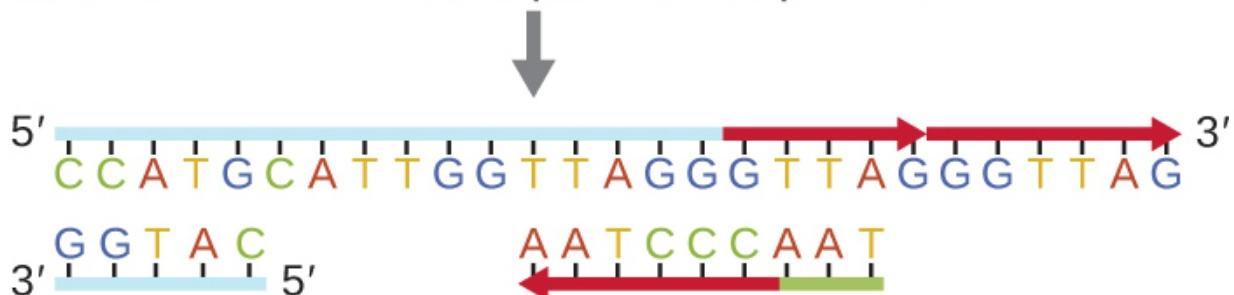
Telomerase has an associated RNA that complements the 3' overhang at the end of the chromosome.



The RNA template is used to synthesize the complementary strand.



Telomerase shifts, and the process is repeated.



Primase and DNA polymerase synthesize the complementary strand.

In eukaryotes, the ends of the linear chromosomes are maintained by

the action of the telomerase enzyme.

Comparison of Bacterial and Eukaryotic Replication		
Property	Bacteria	Eukaryotes
Genome structure	Single circular chromosome	Multiple linear chromosomes
Number of origins per chromosome	Single	Multiple
Rate of replication	1000 nucleotides per second	100 nucleotides per second
Telomerase	Not present	Present
RNA primer removal	DNA pol I	RNase H
Strand elongation	DNA pol III	pol δ , pol ϵ

Note:



This [animation](#) illustrates the process of DNA replication.

Note:

- How does the origin of replication differ between eukaryotes and prokaryotes?
- What polymerase enzymes are responsible for DNA synthesis during eukaryotic replication?
- What is found at the ends of the chromosomes in eukaryotes and why?

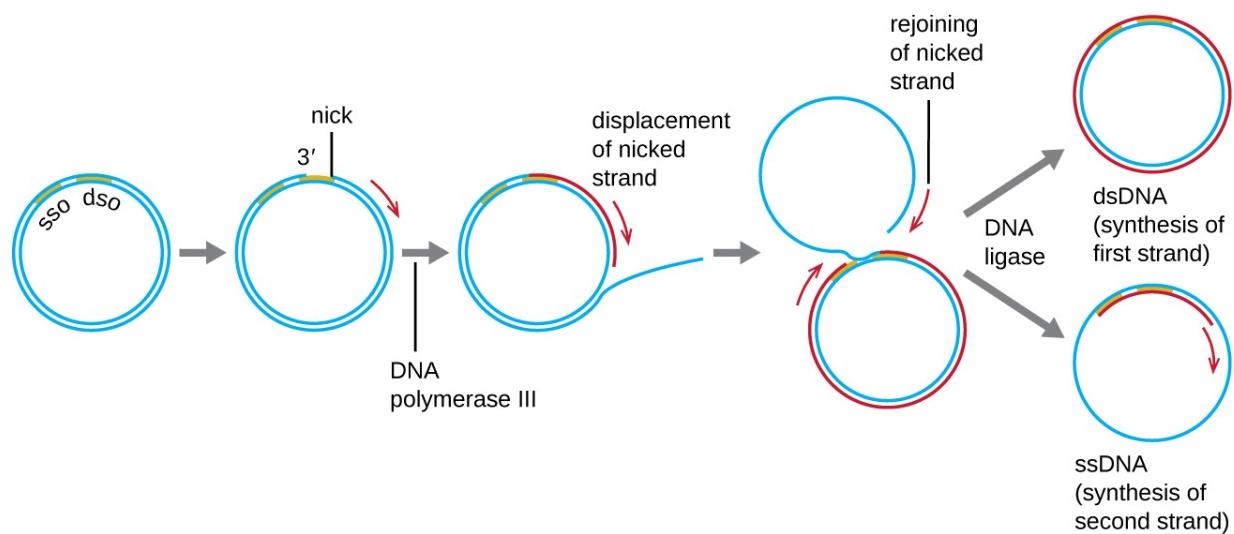
DNA Replication of Extrachromosomal Elements: Plasmids and Viruses

To copy their nucleic acids, plasmids and viruses frequently use variations on the pattern of DNA replication described for prokaryote genomes. For more information on the wide range of viral replication strategies, see [The Viral Life Cycle](#).

Rolling Circle Replication

Whereas many bacterial plasmids (see [Unique Characteristics of Prokaryotic Cells](#)) replicate by a process similar to that used to copy the

bacterial chromosome, other plasmids, several bacteriophages, and some viruses of eukaryotes use **rolling circle replication** ([\[link\]](#)). The circular nature of plasmids and the circularization of some viral genomes on infection make this possible. Rolling circle replication begins with the enzymatic nicking of one strand of the double-stranded circular molecule at the double-stranded origin (dso) site. In bacteria, DNA polymerase III binds to the 3'-OH group of the nicked strand and begins to unidirectionally replicate the DNA using the un-nicked strand as a template, displacing the nicked strand as it does so. Completion of DNA replication at the site of the original nick results in full displacement of the nicked strand, which may then recircularize into a single-stranded DNA molecule. RNA primase then synthesizes a primer to initiate DNA replication at the single-stranded origin (sso) site of the single-stranded DNA (ssDNA) molecule, resulting in a double-stranded DNA (dsDNA) molecule identical to the other circular DNA molecule.



The process of rolling circle replication results in the synthesis of a single new copy of the circular DNA molecule, as shown here.

Note:

- Is there a lagging strand in rolling circle replication? Why or why not?

Key Concepts and Summary

- The DNA replication process is **semiconservative**, which results in two DNA molecules, each having one parental strand of DNA and one newly synthesized strand.
- In bacteria, the **initiation of replication** occurs at the **origin of replication**, where **supercoiled** DNA is unwound by **DNA gyrase**, made single-stranded by **helicase**, and bound by **single-stranded binding protein** to maintain its single-stranded state. **Primase** synthesizes a short RNA **primer**, providing a free 3'-OH group to which **DNA polymerase III** can add DNA nucleotides.
- During **elongation**, the **leading strand** of DNA is synthesized continuously from a single primer. The **lagging strand** is synthesized discontinuously in short **Okazaki fragments**, each requiring its own primer. The RNA primers are removed and replaced with DNA nucleotides by bacterial **DNA polymerase I**, and **DNA ligase** seals the gaps between these fragments.
- **Termination** of replication in bacteria involves the resolution of circular DNA concatemers by topoisomerase IV to release the two copies of the circular chromosome.
- Eukaryotes typically have multiple linear chromosomes, each with multiple origins of replication. Overall, replication in eukaryotes is similar to that in prokaryotes.
- The linear nature of eukaryotic chromosomes necessitates **telomeres** to protect genes near the end of the chromosomes. **Telomerase** extends telomeres, preventing their degradation, in some cell types.
- **Rolling circle replication** is a type of rapid unidirectional DNA synthesis of a circular DNA molecule used for the replication of some plasmids.

Critical Thinking

Exercise:

Problem:

Review [\[link\]](#) and [\[link\]](#). Why was it important that Meselson and Stahl continue their experiment to at least two rounds of replication after isotopic labeling of the starting DNA with ^{15}N , instead of stopping the experiment after only one round of replication?

Exercise:

Problem:

If deoxyribonucleotides that lack the 3'-OH groups are added during the replication process, what do you expect will occur?

RNA Transcription

LEARNING OBJECTIVES

- Explain how RNA is synthesized using DNA as a template
- Distinguish between transcription in prokaryotes and eukaryotes

During the process of **transcription**, the information encoded within the DNA sequence of one or more genes is transcribed into a strand of RNA, also called an **RNA transcript**. The resulting single-stranded RNA molecule, composed of ribonucleotides containing the bases adenine (A), cytosine (C), guanine (G), and uracil (U), acts as a mobile molecular copy of the original DNA sequence. Transcription in prokaryotes and in eukaryotes requires the DNA double helix to partially unwind in the region of RNA synthesis. The unwound region is called a **transcription bubble**. Transcription of a particular gene always proceeds from one of the two DNA strands that acts as a template, the so-called **antisense strand**. The RNA product is complementary to the template strand of DNA and is almost identical to the nontemplate DNA strand, or the **sense strand**. The only difference is that in RNA, all of the T nucleotides are replaced with U nucleotides; during RNA synthesis, U is incorporated when there is an A in the complementary antisense strand.

Transcription in Bacteria

Bacteria use the same RNA polymerase to transcribe all of their genes. Like DNA polymerase, **RNA polymerase** adds nucleotides one by one to the 3'-OH group of the growing nucleotide chain. One critical difference in

activity between DNA polymerase and RNA polymerase is the requirement for a 3'-OH onto which to add nucleotides: DNA polymerase requires such a 3'-OH group, thus necessitating a primer, whereas RNA polymerase does not. During transcription, a ribonucleotide complementary to the DNA template strand is added to the growing RNA strand and a covalent phosphodiester bond is formed by dehydration synthesis between the new nucleotide and the last one added. In *E. coli*, RNA polymerase comprises six polypeptide subunits, five of which compose the polymerase core enzyme responsible for adding RNA nucleotides to a growing strand. The sixth subunit is known as sigma (σ). The σ factor enables RNA polymerase to bind to a specific promoter, thus allowing for the transcription of various genes. There are various σ factors that allow for transcription of various genes.

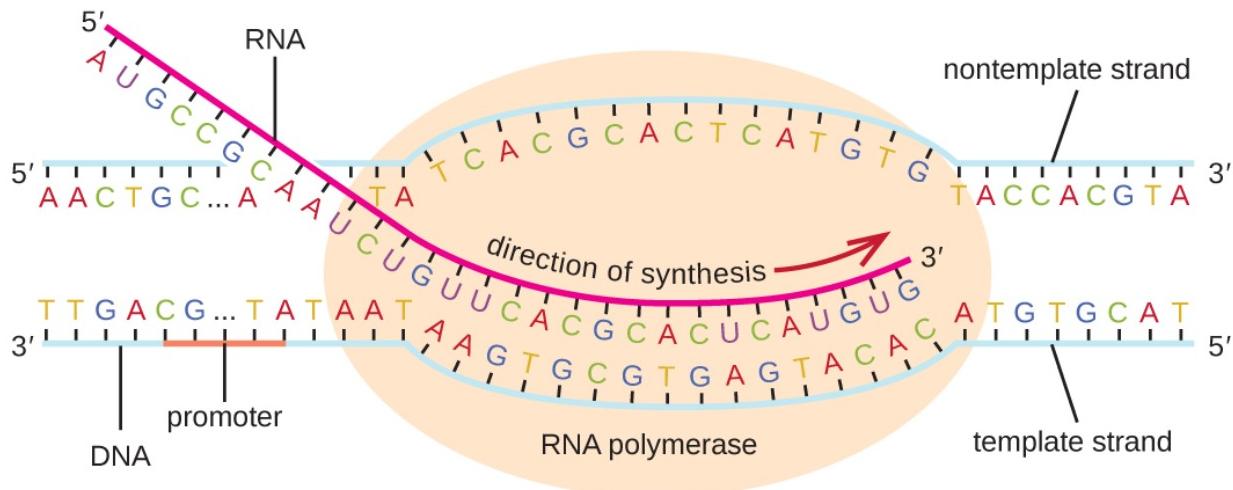
Initiation

The **initiation of transcription** begins at a **promoter**, a DNA sequence onto which the transcription machinery binds and initiates transcription. The nucleotide pair in the DNA double helix that corresponds to the site from which the first 5' RNA nucleotide is transcribed is the initiation site. Nucleotides preceding the initiation site are designated “upstream,” whereas nucleotides following the initiation site are called “downstream” nucleotides. In most cases, promoters are located just upstream of the genes they regulate. Although promoter sequences vary among bacterial genomes, a few elements are conserved. At the -10 and -35 positions within the DNA prior to the initiation site (designated +1), there are two promoter consensus sequences, or regions that are similar across all promoters and across various bacterial species. The -10 consensus sequence, called the TATA box, is TATAAT. The -35 sequence is recognized and bound by σ .

Elongation

The **elongation in transcription** phase begins when the σ subunit dissociates from the polymerase, allowing the core enzyme to synthesize

RNA complementary to the DNA template in a 5' to 3' direction at a rate of approximately 40 nucleotides per second. As elongation proceeds, the DNA is continuously unwound ahead of the core enzyme and rewound behind it ([\[link\]](#)).



During elongation, the bacterial RNA polymerase tracks along the DNA template, synthesizes mRNA in the 5' to 3' direction, and unwinds and rewinds the DNA as it is read.

Termination

Once a gene is transcribed, the bacterial polymerase must dissociate from the DNA template and liberate the newly made RNA. This is referred to as **termination of transcription**. The DNA template includes repeated nucleotide sequences that act as termination signals, causing RNA polymerase to stall and release from the DNA template, freeing the RNA transcript.

Note:

- Where does σ factor of RNA polymerase bind DNA to start transcription?
- What occurs to initiate the polymerization activity of RNA polymerase?
- Where does the signal to end transcription come from?

Transcription in Eukaryotes

Prokaryotes and eukaryotes perform fundamentally the same process of transcription, with a few significant differences (see [[link](#)]). Eukaryotes use three different polymerases, RNA polymerases I, II, and III, all structurally distinct from the bacterial RNA polymerase. Each transcribes a different subset of genes. Interestingly, archaea contain a single RNA polymerase that is more closely related to eukaryotic RNA polymerase II than to its bacterial counterpart. Eukaryotic mRNAs are also usually monocistronic, meaning that they each encode only a single polypeptide, whereas prokaryotic mRNAs of bacteria and archaea are commonly **polycistronic**, meaning that they encode multiple polypeptides.

The most important difference between prokaryotes and eukaryotes is the latter's membrane-bound nucleus, which influences the ease of use of RNA molecules for protein synthesis. With the genes bound in a nucleus, the eukaryotic cell must transport protein-encoding RNA molecules to the cytoplasm to be translated. Protein-encoding **primary transcripts**, the RNA molecules directly synthesized by RNA polymerase, must undergo several processing steps to protect these RNA molecules from degradation during the time they are transferred from the nucleus to the cytoplasm and translated into a protein. For example, eukaryotic mRNAs may last for several hours, whereas the typical prokaryotic mRNA lasts no more than 5 seconds.

The primary transcript (also called pre-mRNA) is first coated with RNA-stabilizing proteins to protect it from degradation while it is processed and

exported out of the nucleus. The first type of processing begins while the primary transcript is still being synthesized; a special 7-methylguanosine nucleotide, called the **5' cap**, is added to the 5' end of the growing transcript. In addition to preventing degradation, factors involved in subsequent protein synthesis recognize the cap, which helps initiate translation by ribosomes. Once elongation is complete, another processing enzyme then adds a string of approximately 200 adenine nucleotides to the 3' end, called the **poly-A tail**. This modification further protects the pre-mRNA from degradation and signals to cellular factors that the transcript needs to be exported to the cytoplasm.

Eukaryotic genes that encode polypeptides are composed of coding sequences called **exons** (*ex-on* signifies that they are *expressed*) and intervening sequences called **introns** (*int-ron* denotes their *intervening* role). Transcribed RNA sequences corresponding to introns do not encode regions of the functional polypeptide and are removed from the pre-mRNA during processing. It is essential that all of the intron-encoded RNA sequences are completely and precisely removed from a pre-mRNA before protein synthesis so that the exon-encoded RNA sequences are properly joined together to code for a functional polypeptide. If the process errs by even a single nucleotide, the sequences of the rejoined exons would be shifted, and the resulting polypeptide would be nonfunctional. The process of removing intron-encoded RNA sequences and reconnecting those encoded by exons is called **RNA splicing** and is facilitated by the action of a **spliceosome** containing small nuclear ribonucleo proteins (snRNPs). Intron-encoded RNA sequences are removed from the pre-mRNA while it is still in the nucleus. Although they are not translated, introns appear to have various functions, including gene regulation and mRNA transport. On completion of these modifications, the mature transcript, the mRNA that encodes a polypeptide, is transported out of the nucleus, destined for the cytoplasm for translation. Introns can be spliced out differently, resulting in various exons being included or excluded from the final mRNA product. This process is known as alternative splicing. The advantage of alternative splicing is that different types of mRNA transcripts can be generated, all derived from the same DNA sequence. In recent years, it has been shown that some archaea also have the ability to splice their pre-mRNA.

Comparison of Transcription in Bacteria Versus Eukaryotes

Property	Bacteria	Eukaryotes
Number of polypeptides encoded per mRNA	Monocistronic or polycistronic	Exclusively monocistronic
Strand elongation	core + σ = holoenzyme	RNA polymerases I, II, or III
Addition of 5' cap	No	Yes
Addition of 3' poly-A tail	No	Yes
Splicing of pre-mRNA	No	Yes

Note:



Visualize how [mRNA splicing](#) happens by watching the process in action in this video. See how introns are removed during [RNA splicing](#) here.

Note:

- In eukaryotic cells, how is the RNA transcript from a gene for a protein modified after it is transcribed?
- Do exons or introns contain information for protein sequences?

Key Concepts and Summary

- During **transcription**, the information encoded in DNA is used to make RNA.
- **RNA polymerase** synthesizes RNA, using the antisense strand of the DNA as template by adding complementary RNA nucleotides to the 3' end of the growing strand.
- RNA polymerase binds to DNA at a sequence called a **promoter** during the **initiation of transcription**.
- Genes encoding proteins of related functions are frequently transcribed under the control of a single promoter in prokaryotes, resulting in the formation of a **polycistronic mRNA** molecule that encodes multiple polypeptides.
- Unlike DNA polymerase, RNA polymerase does not require a 3'-OH group to add nucleotides, so a **primer** is not needed during initiation.
- **Termination of transcription** in bacteria occurs when the RNA polymerase encounters specific DNA sequences that lead to stalling of the polymerase. This results in release of RNA polymerase from the DNA template strand, freeing the **RNA transcript**.
- Eukaryotes have three different RNA polymerases. Eukaryotes also have monocistronic mRNA, each encoding only a single polypeptide.
- Eukaryotic primary transcripts are processed in several ways, including the addition of a **5' cap** and a **3'-poly-A tail**, as well as **splicing**, to generate a mature mRNA molecule that can be transported out of the nucleus and that is protected from degradation.

Protein Translation

LEARNING OBJECTIVES

- Describe the genetic code and explain why it is considered almost universal
- Explain the process of translation and the functions of the molecular machinery of translation
- Compare translation in eukaryotes and prokaryotes

The synthesis of proteins consumes more of a cell's energy than any other metabolic process. In turn, proteins account for more mass than any other macromolecule of living organisms. They perform virtually every function of a cell, serving as both functional (e.g., enzymes) and structural elements. The process of **translation**, or **protein synthesis**, the second part of gene expression, involves the decoding by a ribosome of an mRNA message into a polypeptide product.

The Genetic Code

Translation of the mRNA template converts nucleotide-based genetic information into the “language” of amino acids to create a protein product. A protein sequence consists of 20 commonly occurring amino acids. Each amino acid is defined within the mRNA by a triplet of nucleotides called a **codon**. The relationship between an mRNA codon and its corresponding amino acid is called the **genetic code**.

The three-nucleotide code means that there is a total of 64 possible combinations (4^3 , with four different nucleotides possible at each of the

three different positions within the codon). This number is greater than the number of amino acids and a given amino acid is encoded by more than one codon ([\[link\]](#)). This redundancy in the genetic code is called **degeneracy**. Typically, whereas the first two positions in a codon are important for determining which amino acid will be incorporated into a growing polypeptide, the third position, called the **wobble position**, is less critical. In some cases, if the nucleotide in the third position is changed, the same amino acid is still incorporated.

Whereas 61 of the 64 possible triplets code for amino acids, three of the 64 codons do not code for an amino acid; they terminate protein synthesis, releasing the polypeptide from the translation machinery. These are called **stop codons** or **nonsense codons**. Another codon, AUG, also has a special function. In addition to specifying the amino acid methionine, it also typically serves as the **start codon** to initiate translation. The **reading frame**, the way nucleotides in mRNA are grouped into codons, for translation is set by the AUG start codon near the 5' end of the mRNA. Each set of three nucleotides following this start codon is a codon in the mRNA message.

The genetic code is nearly universal. With a few exceptions, virtually all species use the same genetic code for protein synthesis, which is powerful evidence that all extant life on earth shares a common origin. However, unusual amino acids such as selenocysteine and pyrrolysine have been observed in archaea and bacteria. In the case of selenocysteine, the codon used is UGA (normally a stop codon). However, UGA can encode for selenocysteine using a stem-loop structure (known as the selenocysteine insertion sequence, or SECIS element), which is found at the 3' untranslated region of the mRNA. Pyrrolysine uses a different stop codon, UAG. The incorporation of pyrrolysine requires the *pylS* gene and a unique transfer RNA (tRNA) with a CUA anticodon.

	second letter				
	U	C	A	G	
first letter	UUU UUC UUA UUG } Phe	UCU UCC UCA UCG } Ser	UAU UAC UAA stop UAG stop } Tyr	UGU UGC UGA stop UGG Trp } Cys	U C A G
C	CUU CUC CUA CUG } Leu	CCU CCC CCA CCG } Pro	CAU CAC CAA CAG } His	CGU CGC CGA CGG } Arg	U C A G
A	AUU AUC AUA AUG Met	ACU ACC ACA ACG } Thr	AAU AAC AAA AAG } Asn	AGU AGC AGA AGG } Ser	U C A G
G	GUU GUC GUA GUG } Val	GCU GCC GCA GCG } Ala	GAU GAC GAA GAG } Asp	GGU GGC GGA GGG } Gly	U C A G

This figure shows the genetic code for translating each nucleotide triplet in mRNA into an amino acid or a termination signal in a nascent protein. The first letter of a codon is shown vertically on the left, the second letter horizontally across the top, and the third letter vertically on the right. (credit: modification of work by National Institutes of Health)

Note:

- How many bases are in each codon?

- What amino acid is coded for by the codon AAU?
- What happens when a stop codon is reached?

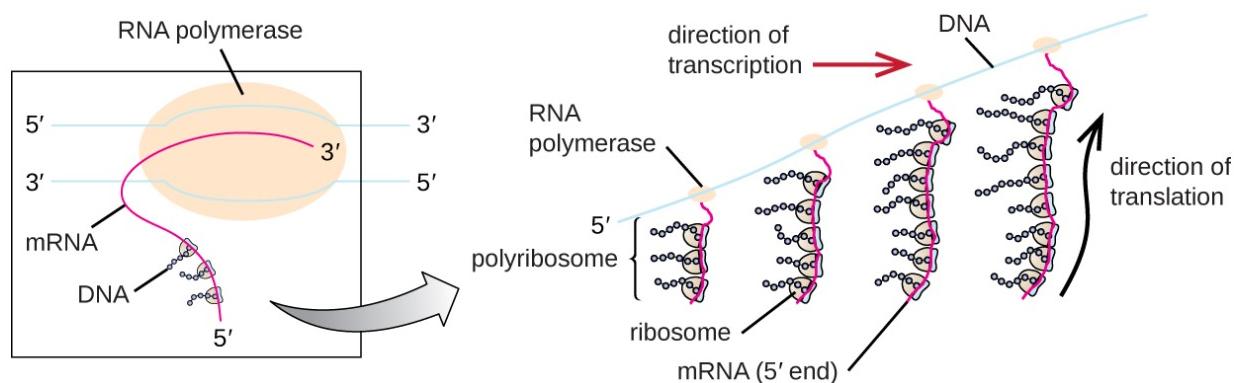
The Protein Synthesis Machinery

In addition to the mRNA template, many molecules and macromolecules contribute to the process of translation. The composition of each component varies across taxa; for instance, ribosomes may consist of different numbers of ribosomal RNAs (rRNAs) and polypeptides depending on the organism. However, the general structures and functions of the protein synthesis machinery are comparable from bacteria to human cells. Translation requires the input of an mRNA template, ribosomes, tRNAs, and various enzymatic factors.

Ribosomes

A ribosome is a complex macromolecule composed of catalytic rRNAs (called ribozymes) and structural rRNAs, as well as many distinct polypeptides. Mature rRNAs make up approximately 50% of each ribosome. Prokaryotes have 70S ribosomes, whereas eukaryotes have 80S ribosomes in the cytoplasm and rough endoplasmic reticulum, and 70S ribosomes in mitochondria and chloroplasts. Ribosomes dissociate into large and small subunits when they are not synthesizing proteins and reassociate during the **initiation of translation**. In *E. coli*, the small subunit is described as 30S (which contains the 16S rRNA subunit), and the large subunit is 50S (which contains the 5S and 23S rRNA subunits), for a total of 70S (Svedberg units are not additive). Eukaryote ribosomes have a small 40S subunit (which contains the 18S rRNA subunit) and a large 60S subunit (which contains the 5S, 5.8S and 28S rRNA subunits), for a total of 80S. The small subunit is responsible for binding the mRNA template, whereas the large subunit binds tRNAs (discussed in the next subsection).

Each mRNA molecule is simultaneously translated by many ribosomes, all synthesizing protein in the same direction: reading the mRNA from 5' to 3' and synthesizing the polypeptide from the N terminus to the C terminus. The complete structure containing an mRNA with multiple associated ribosomes is called a **polyribosome** (or **polysome**). In both bacteria and archaea, before transcriptional termination occurs, each protein-encoding transcript is already being used to begin synthesis of numerous copies of the encoded polypeptide(s) because the processes of transcription and translation can occur concurrently, forming polyribosomes ([\[link\]](#)). The reason why transcription and translation can occur simultaneously is because both of these processes occur in the same 5' to 3' direction, they both occur in the cytoplasm of the cell, and because the RNA transcript is not processed once it is transcribed. This allows a prokaryotic cell to respond to an environmental signal requiring new proteins very quickly. In contrast, in eukaryotic cells, simultaneous transcription and translation is not possible. Although polyribosomes also form in eukaryotes, they cannot do so until RNA synthesis is complete and the RNA molecule has been modified and transported out of the nucleus.



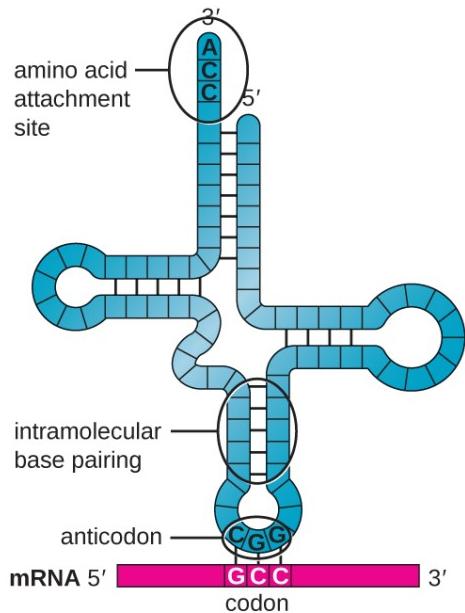
In prokaryotes, multiple RNA polymerases can transcribe a single bacterial gene while numerous ribosomes concurrently translate the mRNA transcripts into polypeptides. In this way, a specific protein can rapidly reach a high concentration in the bacterial cell.

Transfer RNAs

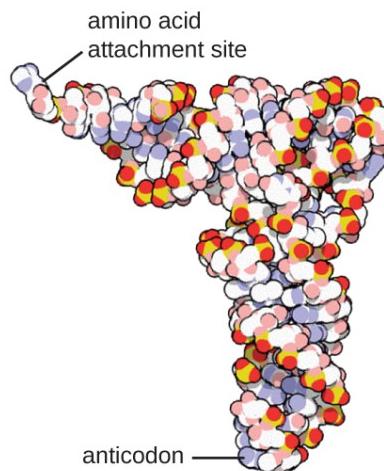
Transfer RNAs (tRNAs) are structural RNA molecules and, depending on the species, many different types of tRNAs exist in the cytoplasm. Bacterial species typically have between 60 and 90 types. Serving as adaptors, each tRNA type binds to a specific codon on the mRNA template and adds the corresponding amino acid to the polypeptide chain. Therefore, tRNAs are the molecules that actually “translate” the language of RNA into the language of proteins. As the adaptor molecules of translation, it is surprising that tRNAs can fit so much specificity into such a small package. The tRNA molecule interacts with three factors: aminoacyl tRNA synthetases, ribosomes, and mRNA.

Mature tRNAs take on a three-dimensional structure when complementary bases exposed in the single-stranded RNA molecule hydrogen bond with each other ([\[link\]](#)). This shape positions the amino-acid binding site, called the **CCA amino acid binding end**, which is a cytosine-cytosine-adenine sequence at the 3' end of the tRNA, and the **anticodon** at the other end. The anticodon is a three-nucleotide sequence that bonds with an mRNA codon through complementary base pairing.

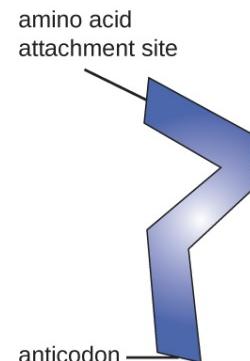
An amino acid is added to the end of a tRNA molecule through the process of tRNA “charging,” during which each tRNA molecule is linked to its correct or **cognate amino acid** by a group of enzymes called **aminoacyl tRNA synthetases**. At least one type of aminoacyl tRNA synthetase exists for each of the 20 amino acids. During this process, the amino acid is first activated by the addition of adenosine monophosphate (AMP) and then transferred to the tRNA, making it a **charged tRNA**, and AMP is released.



(a)



(b)



(c)

(a) After folding caused by intramolecular base pairing, a tRNA molecule has one end that contains the anticodon, which interacts with the mRNA codon, and the CCA amino acid binding end. (b) A space-filling model is helpful for visualizing the three-dimensional shape of tRNA. (c) Simplified models are useful when drawing complex processes such as protein synthesis.

Note:

- Describe the structure and composition of the prokaryotic ribosome.
- In what direction is the mRNA template read?
- Describe the structure and function of a tRNA.

The Mechanism of Protein Synthesis

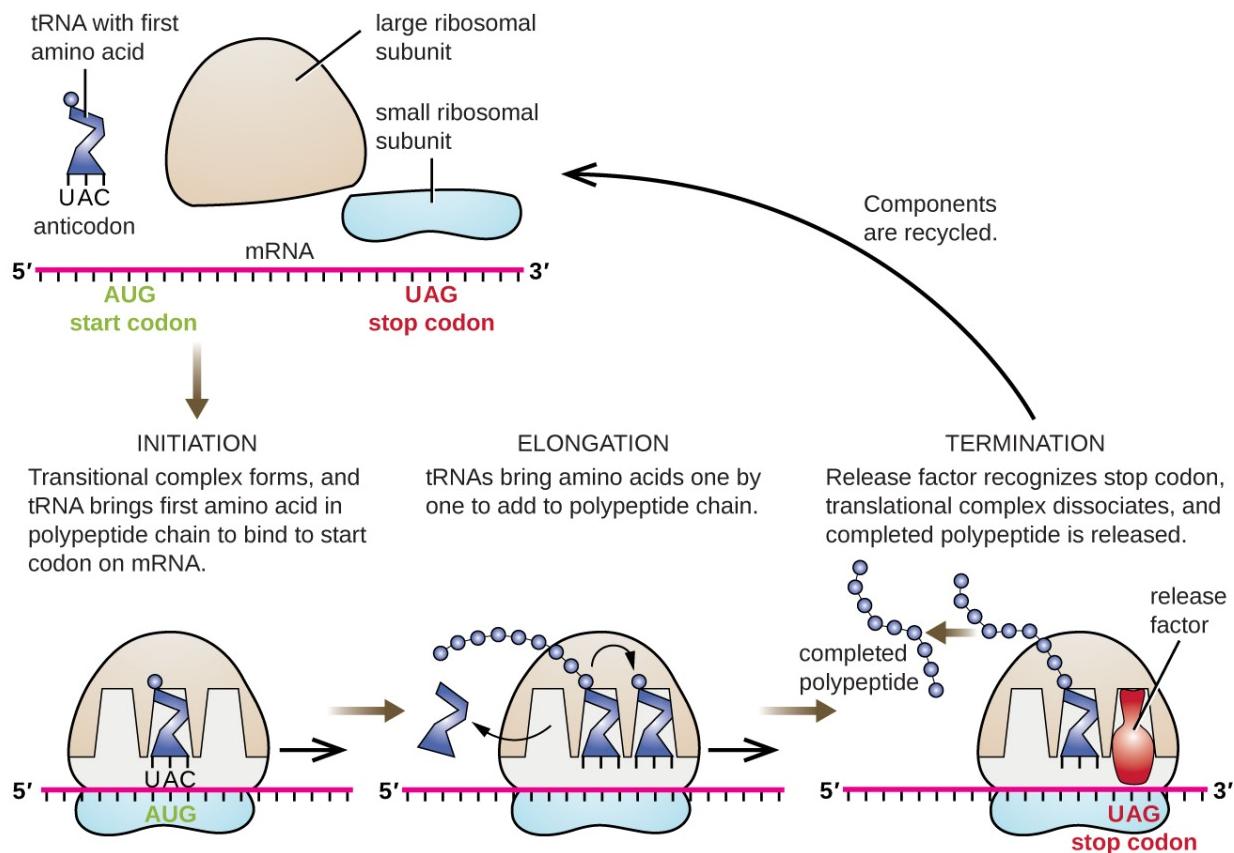
Translation is similar in prokaryotes and eukaryotes. Here we will explore how translation occurs in *E. coli*, a representative prokaryote, and specify any differences between bacterial and eukaryotic translation.

Initiation

The **initiation of protein synthesis** begins with the formation of an initiation complex. In *E. coli*, this complex involves the small 30S ribosome, the mRNA template, three **initiation factors** that help the ribosome assemble correctly, guanosine triphosphate (GTP) that acts as an energy source, and a special initiator tRNA carrying *N*-formyl-methionine (fMet-tRNA^{fMet}) ([\[link\]](#)). The initiator tRNA interacts with the start codon AUG of the mRNA and carries a formylated methionine (fMet). Because of its involvement in initiation, fMet is inserted at the beginning (N terminus) of every polypeptide chain synthesized by *E. coli*. In *E. coli* mRNA, a leader sequence upstream of the first AUG codon, called the Shine-Dalgarno sequence (also known as the ribosomal binding site AGGAGG), interacts through complementary base pairing with the rRNA molecules that compose the ribosome. This interaction anchors the 30S ribosomal subunit at the correct location on the mRNA template. At this point, the 50S ribosomal subunit then binds to the initiation complex, forming an intact ribosome.

In eukaryotes, initiation complex formation is similar, with the following differences:

- The initiator tRNA is a different specialized tRNA carrying methionine, called Met-tRNA_i
- Instead of binding to the mRNA at the Shine-Dalgarno sequence, the eukaryotic initiation complex recognizes the 5' cap of the eukaryotic mRNA, then tracks along the mRNA in the 5' to 3' direction until the AUG start codon is recognized. At this point, the 60S subunit binds to the complex of Met-tRNA_i, mRNA, and the 40S subunit.



Translation in bacteria begins with the formation of the initiation complex, which includes the small ribosomal subunit, the mRNA, the initiator tRNA carrying N-formyl-methionine, and initiation factors.

Then the 50S subunit binds, forming an intact ribosome.

Elongation

In prokaryotes and eukaryotes, the basics of **elongation of translation** are the same. In *E. coli*, the binding of the 50S ribosomal subunit to produce the intact ribosome forms three functionally important ribosomal sites: The **A (aminoacyl) site** binds incoming charged aminoacyl tRNAs. The **P (peptidyl) site** binds charged tRNAs carrying amino acids that have formed peptide bonds with the growing polypeptide chain but have not yet dissociated from their corresponding tRNA. The **E (exit) site** releases

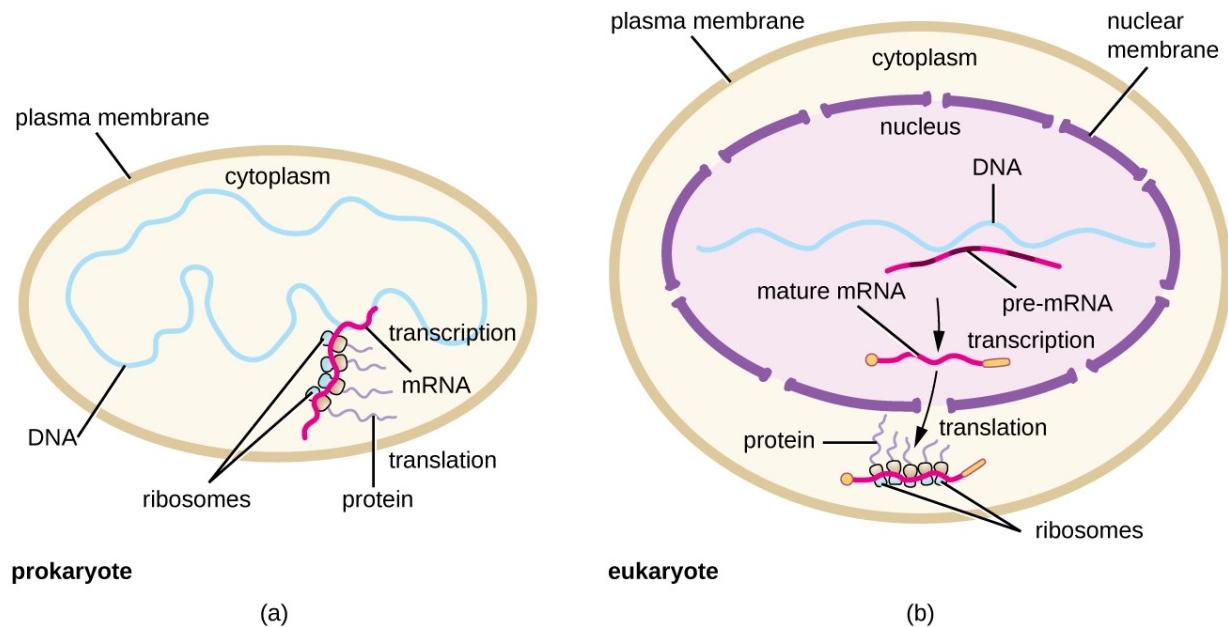
dissociated tRNAs so that they can be recharged with free amino acids. There is one notable exception to this assembly line of tRNAs: During initiation complex formation, bacterial fMet-tRNA^{fMet} or eukaryotic Met-tRNA_i enters the P site directly without first entering the A site, providing a free A site ready to accept the tRNA corresponding to the first codon after the AUG.

Elongation proceeds with single-codon movements of the ribosome each called a translocation event. During each translocation event, the charged tRNAs enter at the A site, then shift to the P site, and then finally to the E site for removal. Ribosomal movements, or steps, are induced by conformational changes that advance the ribosome by three bases in the 3' direction. Peptide bonds form between the amino group of the amino acid attached to the A-site tRNA and the carboxyl group of the amino acid attached to the P-site tRNA. The formation of each peptide bond is catalyzed by **peptidyl transferase**, an RNA-based ribozyme that is integrated into the 50S ribosomal subunit. The amino acid bound to the P-site tRNA is also linked to the growing polypeptide chain. As the ribosome steps across the mRNA, the former P-site tRNA enters the E site, detaches from the amino acid, and is expelled. Several of the steps during elongation, including binding of a charged aminoacyl tRNA to the A site and translocation, require energy derived from GTP hydrolysis, which is catalyzed by specific elongation factors. Amazingly, the *E. coli* translation apparatus takes only 0.05 seconds to add each amino acid, meaning that a 200 amino-acid protein can be translated in just 10 seconds.

Termination

The **termination of translation** occurs when a nonsense codon (UAA, UAG, or UGA) is encountered for which there is no complementary tRNA. On aligning with the A site, these nonsense codons are recognized by release factors in prokaryotes and eukaryotes that result in the P-site amino acid detaching from its tRNA, releasing the newly made polypeptide. The small and large ribosomal subunits dissociate from the mRNA and from each other; they are recruited almost immediately into another translation initiation complex.

In summary, there are several key features that distinguish prokaryotic gene expression from that seen in eukaryotes. These are illustrated in [\[link\]](#) and listed in [\[link\]](#).



- (a) In prokaryotes, the processes of transcription and translation occur simultaneously in the cytoplasm, allowing for a rapid cellular response to an environmental cue. (b) In eukaryotes, transcription is localized to the nucleus and translation is localized to the cytoplasm, separating these processes and necessitating RNA processing for stability.

Comparison of Translation in Bacteria Versus Eukaryotes		
Property	Bacteria	Eukaryotes
Ribosomes	70S <ul style="list-style-type: none"> • 30S (small subunit) with 16S rRNA subunit • 50S (large subunit) with 5S and 23S rRNA subunits 	80S <ul style="list-style-type: none"> • 40S (small subunit) with 18S rRNA subunit • 60S (large subunit) with 5S, 5.8S, and 28S rRNA subunits
Amino acid carried by initiator tRNA	fMet	Met
Shine-Dalgarno sequence in mRNA	Present	Absent
Simultaneous transcription and translation	Yes	No

Protein Targeting, Folding, and Modification

During and after translation, polypeptides may need to be modified before they are biologically active. Post-translational modifications include:

1. removal of translated signal sequences—short tails of amino acids that aid in directing a protein to a specific cellular compartment
2. proper “folding” of the polypeptide and association of multiple polypeptide subunits, often facilitated by chaperone proteins, into a distinct three-dimensional structure
3. proteolytic processing of an inactive polypeptide to release an active protein component, and
4. various chemical modifications (e.g., phosphorylation, methylation, or glycosylation) of individual amino acids.

Note:

- What are the components of the initiation complex for translation in prokaryotes?
- What are two differences between initiation of prokaryotic and eukaryotic translation?
- What occurs at each of the three active sites of the ribosome?
- What causes termination of translation?

Key Concepts and Summary

- In **translation**, polypeptides are synthesized using mRNA sequences and cellular machinery, including tRNAs that match mRNA **codons** to specific amino acids and ribosomes composed of RNA and proteins that catalyze the reaction.
- The **genetic code** is **degenerate** in that several mRNA codons code for the same amino acids. The genetic code is almost universal among living organisms.
- Prokaryotic (70S) and cytoplasmic eukaryotic (80S) ribosomes are each composed of a large subunit and a small subunit of differing sizes between the two groups. Each subunit is composed of rRNA and protein. Organelle ribosomes in eukaryotic cells resemble prokaryotic ribosomes.
- Some 60 to 90 species of tRNA exist in bacteria. Each tRNA has a three-nucleotide **anticodon** as well as a binding site for a **cognate amino acid**. All tRNAs with a specific anticodon will carry the same amino acid.
- **Initiation** of translation occurs when the small ribosomal subunit binds with **initiation factors** and an initiator tRNA at the **start codon** of an mRNA, followed by the binding to the initiation complex of the large ribosomal subunit.
- In prokaryotic cells, the start codon codes for N-formyl-methionine carried by a special initiator tRNA. In eukaryotic cells, the start codon codes for methionine carried by a special initiator tRNA. In addition, whereas ribosomal binding of the mRNA in prokaryotes is facilitated by the Shine-Dalgarno sequence within the mRNA, eukaryotic ribosomes bind to the 5' cap of the mRNA.
- During the **elongation** stage of translation, a **charged tRNA** binds to mRNA in the **A site** of the ribosome; a peptide bond is catalyzed between the two adjacent amino acids, breaking the bond between the first amino acid and its tRNA; the ribosome moves one codon along the mRNA; and the first tRNA is moved from the **P site** of the ribosome to the **E site** and leaves the ribosomal complex.

- **Termination** of translation occurs when the ribosome encounters a **stop codon**, which does not code for a tRNA. Release factors cause the polypeptide to be released, and the ribosomal complex dissociates.
- In prokaryotes, transcription and translation may be coupled, with translation of an mRNA molecule beginning as soon as transcription allows enough mRNA exposure for the binding of a ribosome, prior to transcription termination. Transcription and translation are not coupled in eukaryotes because transcription occurs in the nucleus, whereas translation occurs in the cytoplasm or in association with the rough endoplasmic reticulum.
- Polypeptides often require one or more **post-translational modifications** to become biologically active.

Short Answer

Exercise:

Problem:

Why does translation terminate when the ribosome reaches a stop codon? What happens?

Exercise:

Problem:

How does the process of translation differ between prokaryotes and eukaryotes?

Exercise:

Problem: What is meant by the genetic code being nearly universal?

Exercise:

Problem:

Below is an antisense DNA sequence. Translate the mRNA molecule synthesized using the genetic code, recording the resulting amino acid sequence, indicating the N and C termini.

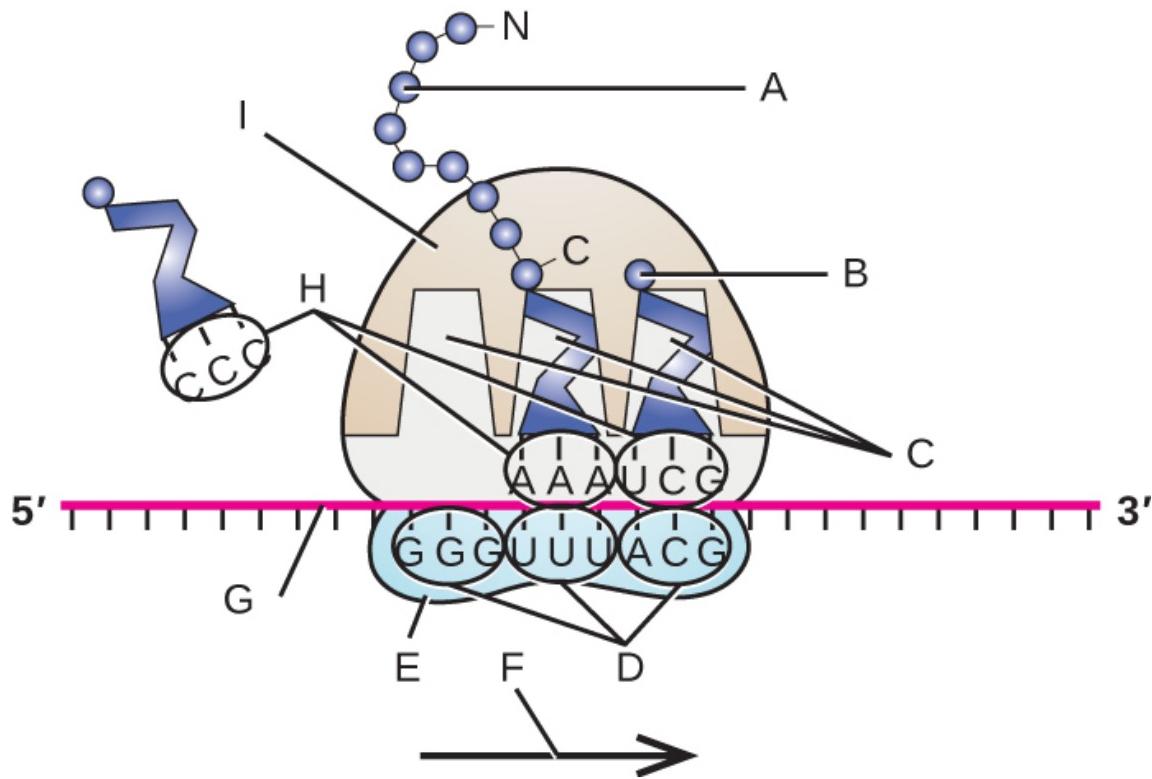
Antisense DNA strand: 3'-T A C T G A C T G A C G A T C-5'

Critical Thinking

Exercise:

Problem:

Label the following in the figure: ribosomal E, P, and A sites; mRNA; codons; anticodons; growing polypeptide; incoming amino acid; direction of translocation; small ribosomal unit; large ribosomal unit.



Exercise:

Problem:

Prior to the elucidation of the genetic code, prominent scientists, including Francis Crick, had predicted that each mRNA codon, coding for one of the 20 amino acids, needed to be at least three nucleotides long. Why is it not possible for codons to be any shorter?

Mutations

LEARNING OBJECTIVES

- Compare point mutations and frameshift mutations
- Describe the differences between missense, nonsense, and silent mutations
- Describe the different mechanisms of DNA repair
- Explain how different mutagens act
- Explain why the Ames test can be used to detect carcinogens
- Analyze sequences of DNA and identify examples of types of mutations
- Discuss possible outcomes of mutations on bacterial cells

A **mutation** is a heritable change in the DNA sequence of an organism. The resulting organism, called a **mutant**, may have a recognizable change in phenotype compared to the **wild type**, which is the phenotype most commonly observed in nature. A change in the DNA sequence is conferred to mRNA through transcription, and may lead to an altered amino acid sequence in a protein on translation. Because proteins carry out the vast majority of cellular functions, a change in amino acid sequence in a protein may lead to an altered phenotype for the cell and organism.

Effects of Mutations on DNA Sequence

There are several types of mutations that are classified according to how the DNA molecule is altered. One type, called a **point mutation**, affects a single base and most commonly occurs when one base is substituted or replaced by another. Mutations also result from the addition of one or more

bases, known as an **insertion**, or the removal of one or more bases, known as a **deletion**.

Note:

- What type of a mutation occurs when a gene has two fewer nucleotides in its sequence?

Effects of Mutations on Protein Structure and Function

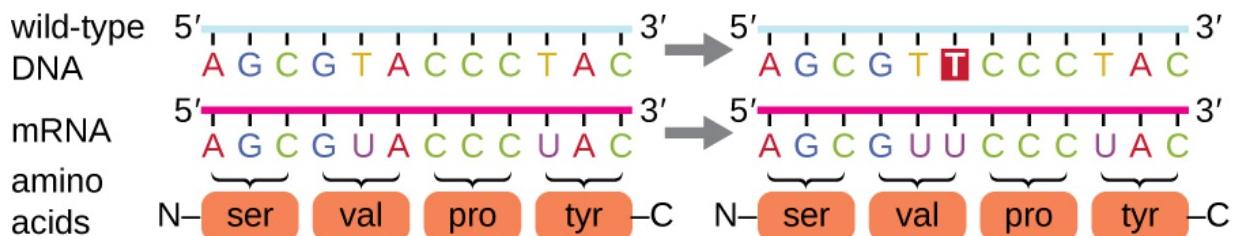
Point mutations may have a wide range of effects on protein function ([\[link\]](#)). As a consequence of the degeneracy of the genetic code, a point mutation will commonly result in the same amino acid being incorporated into the resulting polypeptide despite the sequence change. This change would have no effect on the protein's structure, and is thus called a **silent mutation**. A **missense mutation** results in a different amino acid being incorporated into the resulting polypeptide. The effect of a missense mutation depends on how chemically different the new amino acid is from the wild-type amino acid. The location of the changed amino acid within the protein also is important. For example, if the changed amino acid is part of the enzyme's active site, then the effect of the missense mutation may be significant. Many missense mutations result in proteins that are still functional, at least to some degree. Sometimes the effects of missense mutations may be only apparent under certain environmental conditions; such missense mutations are called **conditional mutations**. Rarely, a missense mutation may be beneficial. Under the right environmental conditions, this type of mutation may give the organism that harbors it a selective advantage. Yet another type of point mutation, called a **nonsense mutation**, converts a codon encoding an amino acid (a sense codon) into a stop codon (a nonsense codon). Nonsense mutations result in the synthesis of proteins that are shorter than the wild type and typically not functional.

Deletions and insertions also cause various effects. Because codons are triplets of nucleotides, insertions or deletions in groups of three nucleotides may lead to the insertion or deletion of one or more amino acids and may not cause significant effects on the resulting protein's functionality.

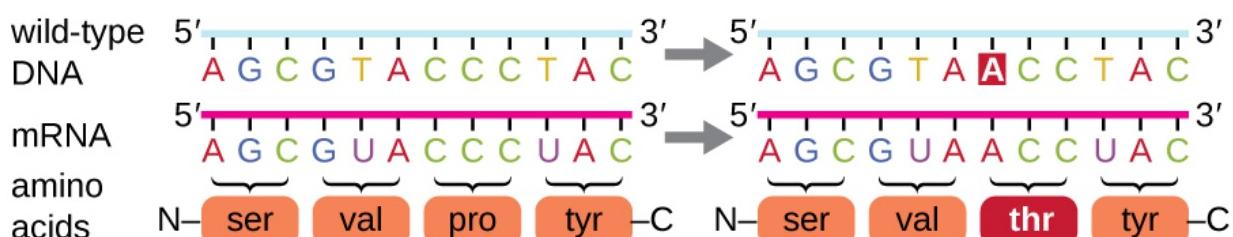
However, **frameshift mutations**, caused by insertions or deletions of a number of nucleotides that are not a multiple of three are extremely problematic because a shift in the reading frame results ([\[link\]](#)). Because ribosomes read the mRNA in triplet codons, frameshift mutations can change every amino acid after the point of the mutation. The new reading frame may also include a stop codon before the end of the coding sequence. Consequently, proteins made from genes containing frameshift mutations are nearly always nonfunctional.

point mutation: substitution of a single base

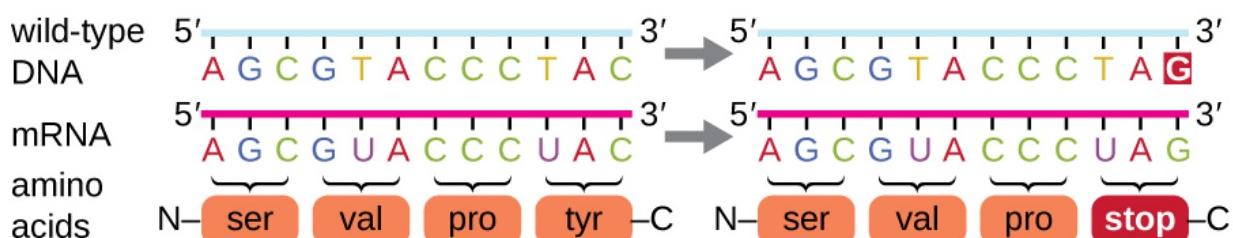
silent: has no effect on the protein sequence



missense: results in an amino acid substitution

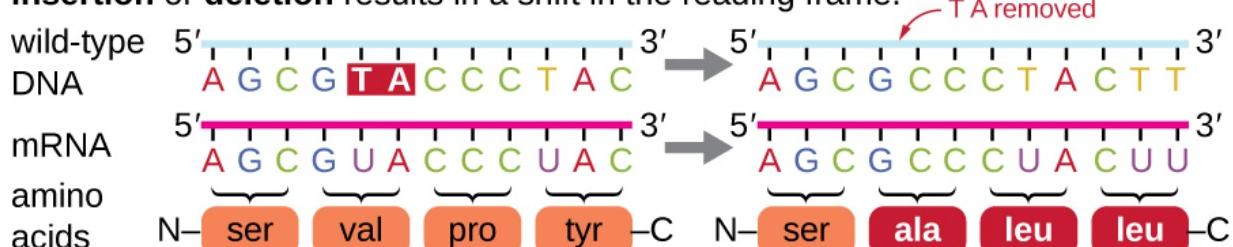


nonsense: substitutes a stop codon for an amino acid



frameshift mutation: insertion or deletion of one or more bases

Insertion or deletion results in a shift in the reading frame.



Mutations can lead to changes in the protein sequence encoded by the DNA.

Note:

- What are the reasons a nucleotide change in a gene for a protein might not have any effect on the phenotype of that gene?
- Is it possible for an insertion of three nucleotides together after the fifth nucleotide in a protein-coding gene to produce a protein that is shorter than normal? How or how not?

Note:**A Beneficial Mutation**

Since the first case of infection with human immunodeficiency virus (HIV) was reported in 1981, nearly 40 million people have died from HIV infection,[footnote] the virus that causes acquired immune deficiency syndrome (AIDS). The virus targets helper T cells that play a key role in bridging the innate and adaptive immune response, infecting and killing cells normally involved in the body's response to infection. There is no cure for HIV infection, but many drugs have been developed to slow or block the progression of the virus. Although individuals around the world may be infected, the highest prevalence among people 15–49 years old is in sub-Saharan Africa, where nearly one person in 20 is infected, accounting for greater than 70% of the infections worldwide[footnote] ([\[link\]](#)). Unfortunately, this is also a part of the world where prevention strategies and drugs to treat the infection are the most lacking.

World Health Organization. “ Global Health Observatory (GHO) Data, HIV/AIDS.” <http://www.who.int/gho/hiv/en/>. Accessed August 5, 2016.

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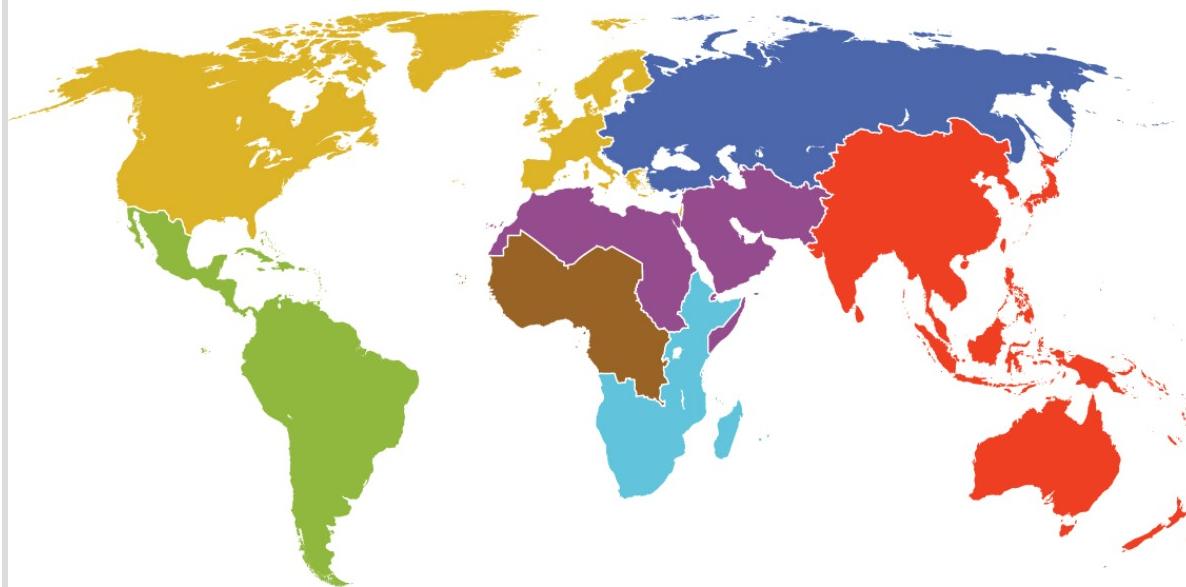
In recent years, scientific interest has been piqued by the discovery of a few individuals from northern Europe who are resistant to HIV infection. In 1998, American geneticist Stephen J. O'Brien at the National Institutes of Health (NIH) and colleagues published the results of their genetic

analysis of more than 4,000 individuals. These indicated that many individuals of Eurasian descent (up to 14% in some ethnic groups) have a deletion mutation, called CCR5-delta 32, in the gene encoding CCR5. CCR5 is a coreceptor found on the surface of T cells that is necessary for many strains of the virus to enter the host cell. The mutation leads to the production of a receptor to which HIV cannot effectively bind and thus blocks viral entry. People homozygous for this mutation have greatly reduced susceptibility to HIV infection, and those who are heterozygous have some protection from infection as well.

It is not clear why people of northern European descent, specifically, carry this mutation, but its prevalence seems to be highest in northern Europe and steadily decreases in populations as one moves south. Research indicates that the mutation has been present since before HIV appeared and may have been selected for in European populations as a result of exposure to the plague or smallpox. This mutation may protect individuals from plague (caused by the bacterium *Yersinia pestis*) and smallpox (caused by the variola virus) because this receptor may also be involved in these diseases. The age of this mutation is a matter of debate, but estimates suggest it appeared between 1875 years to 225 years ago, and may have been spread from Northern Europe through Viking invasions.

This exciting finding has led to new avenues in HIV research, including looking for drugs to block CCR5 binding to HIV in individuals who lack the mutation. Although DNA testing to determine which individuals carry the CCR5-delta 32 mutation is possible, there are documented cases of individuals homozygous for the mutation contracting HIV. For this reason, DNA testing for the mutation is not widely recommended by public health officials so as not to encourage risky behavior in those who carry the mutation. Nevertheless, inhibiting the binding of HIV to CCR5 continues to be a valid strategy for the development of drug therapies for those infected with HIV.

HIV Prevalence Among People Ages 15–49 years, 2015



Global prevalence in 2015

- Middle East and North Africa
- Southern Asia, Australia, and the Pacific
- Western and Central Europe and North America

= 0.8%
= 0.1%
= 0.2%
= 0.3%

- Central and South America = 0.5%
- Eastern Europe and Central Asia = 0.9%
- West and Central Africa = 2.2%
- East and Southern Africa = 7.1%

HIV is highly prevalent in sub-Saharan Africa, but its prevalence is quite low in some other parts of the world.

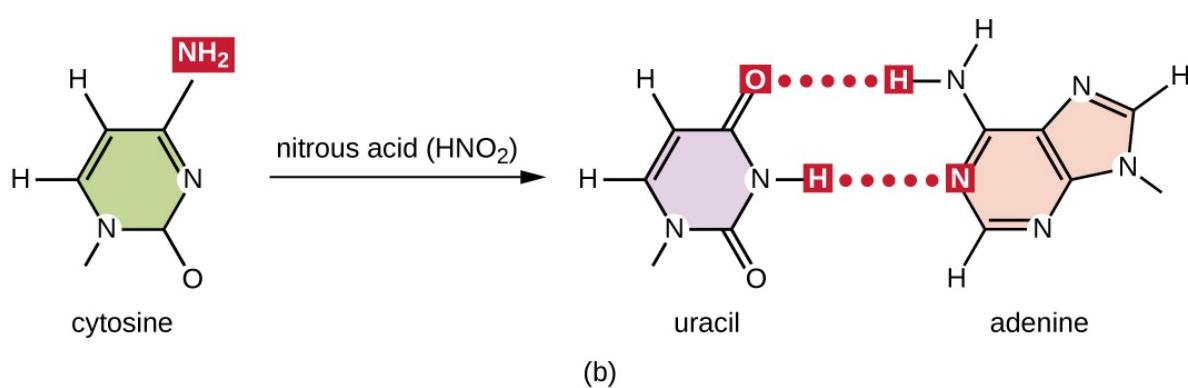
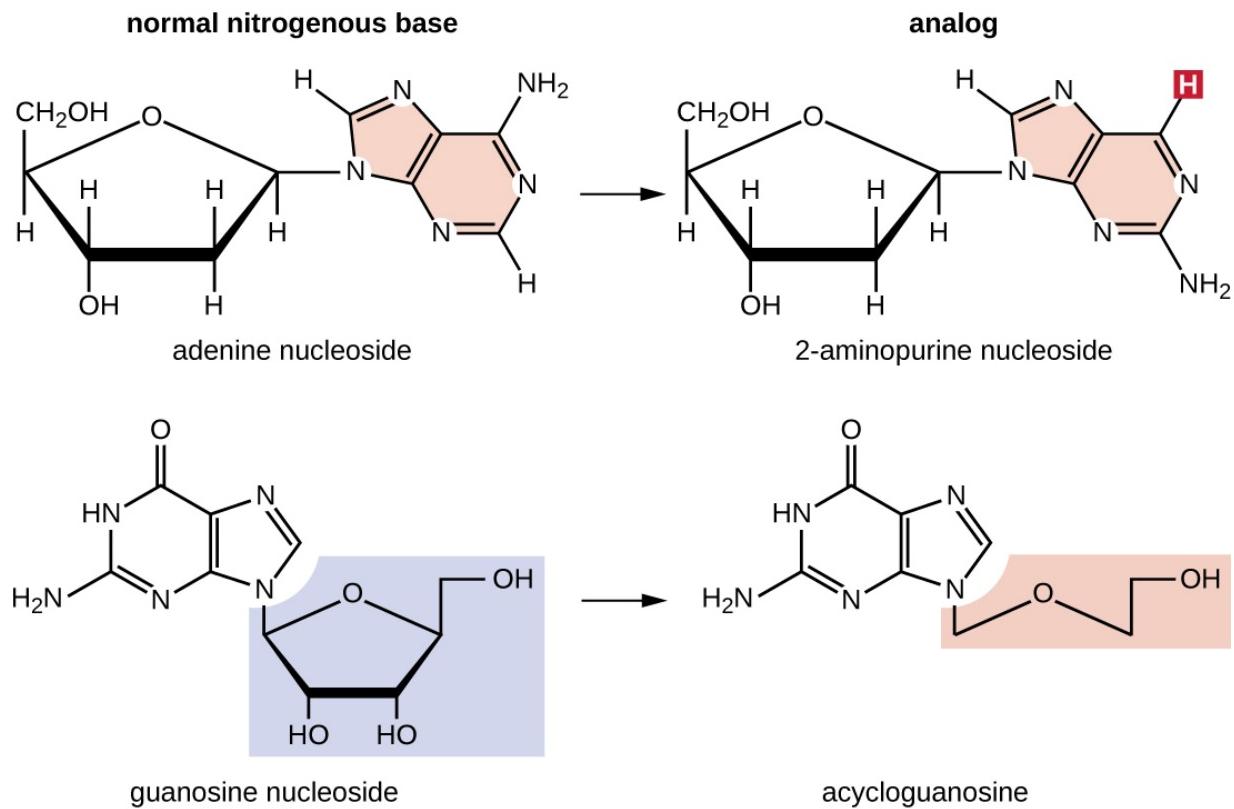
Causes of Mutations

Mistakes in the process of DNA replication can cause **spontaneous mutations** to occur. The error rate of DNA polymerase is one incorrect base per billion base pairs replicated. Exposure to **mutagens** can cause **induced mutations**, which are various types of chemical agents or radiation ([\[link\]](#)). Exposure to a mutagen can increase the rate of mutation more than 1000-fold. Mutagens are often also **carcinogens**, agents that cause cancer. However, whereas nearly all carcinogens are mutagenic, not all mutagens are necessarily carcinogens.

Chemical Mutagens

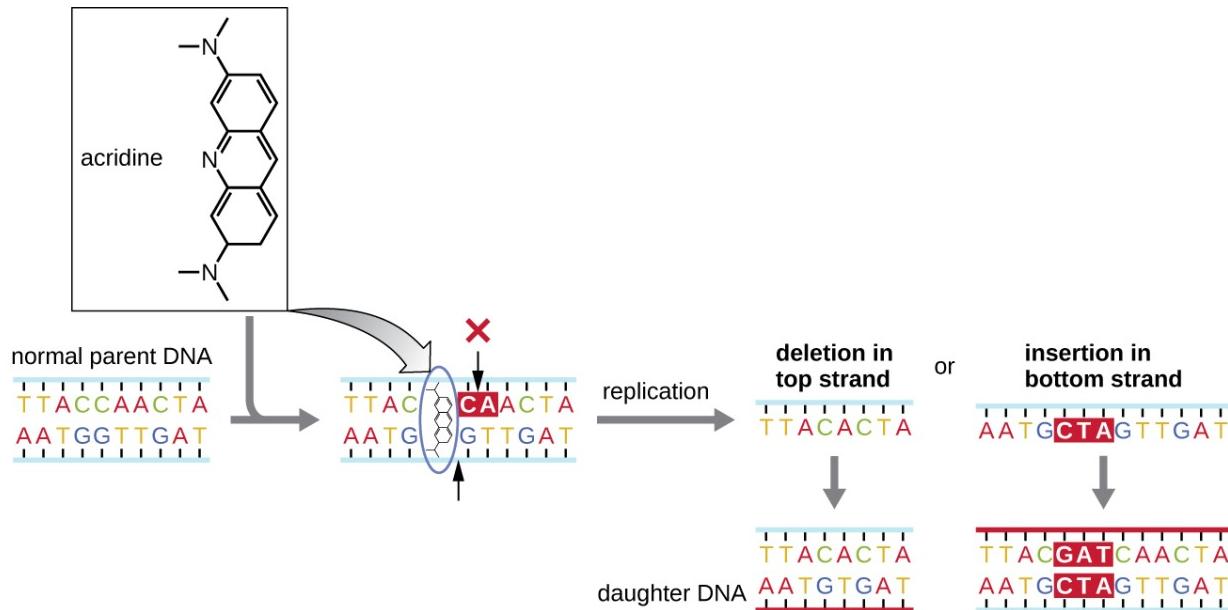
Various types of chemical mutagens interact directly with DNA either by acting as nucleoside analogs or by modifying nucleotide bases. Chemicals called **nucleoside analogs** are structurally similar to normal nucleotide bases and can be incorporated into DNA during replication ([\[link\]](#)). These base analogs induce mutations because they often have different base-pairing rules than the bases they replace. Other chemical mutagens can modify normal DNA bases, resulting in different base-pairing rules. For example, nitrous acid deaminates cytosine, converting it to uracil. Uracil then pairs with adenine in a subsequent round of replication, resulting in the conversion of a GC base pair to an AT base pair. Nitrous acid also deaminates adenine to hypoxanthine, which base pairs with cytosine instead of thymine, resulting in the conversion of a TA base pair to a CG base pair.

Chemical mutagens known as **intercalating agents** work differently. These molecules slide between the stacked nitrogenous bases of the DNA double helix, distorting the molecule and creating atypical spacing between nucleotide base pairs ([\[link\]](#)). As a result, during DNA replication, DNA polymerase may either skip replicating several nucleotides (creating a deletion) or insert extra nucleotides (creating an insertion). Either outcome may lead to a frameshift mutation. Combustion products like polycyclic aromatic hydrocarbons are particularly dangerous intercalating agents that can lead to mutation-caused cancers. The intercalating agents ethidium bromide and acridine orange are commonly used in the laboratory to stain DNA for visualization and are potential mutagens.



(a) 2-aminopurine nucleoside (2AP) structurally is a nucleoside analog to adenine nucleoside, whereas 5-bromouracil (5BU) is a nucleoside analog to thymine nucleoside. 2AP base pairs with C, converting an AT base pair to a GC base pair after several rounds of replication. 5BU pairs with G, converting an AT base pair to a GC base pair after several rounds of replication. (b) Nitrous acid is a different type of chemical mutagen that modifies already existing nucleoside bases like

C to produce U, which base pairs with A. This chemical modification, as shown here, results in converting a CG base pair to a TA base pair.



Intercalating agents, such as acridine, introduce atypical spacing between base pairs, resulting in DNA polymerase introducing either a deletion or an insertion, leading to a potential frameshift mutation.

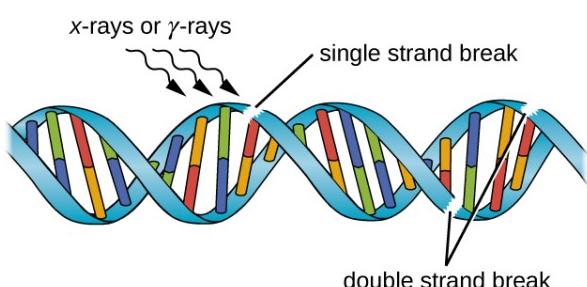
Radiation

Exposure to either ionizing or nonionizing radiation can each induce mutations in DNA, although by different mechanisms. Strong **ionizing radiation** like X-rays and gamma rays can cause single- and double-stranded breaks in the DNA backbone through the formation of hydroxyl radicals on radiation exposure ([\[link\]](#)). Ionizing radiation can also modify bases; for example, the deamination of cytosine to uracil, analogous to the action of nitrous acid.[\[footnote\]](#) Ionizing radiation exposure is used to kill microbes to sterilize medical devices and foods, because of its dramatic

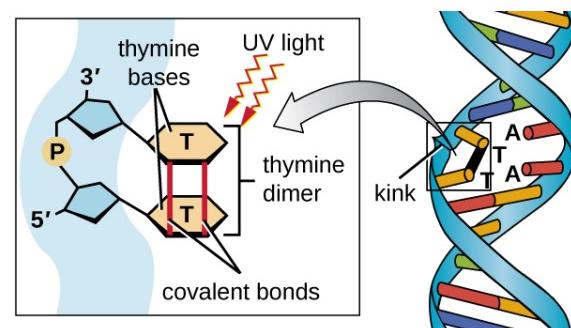
nonspecific effect in damaging DNA, proteins, and other cellular components (see [Using Physical Methods to Control Microorganisms](#)).

K.R. Tindall et al. "Changes in DNA Base Sequence Induced by Gamma-Ray Mutagenesis of Lambda Phage and Prophage." *Genetics* 118 no. 4 (1988):551–560.

Nonionizing radiation, like ultraviolet light, is not energetic enough to initiate these types of chemical changes. However, **nonionizing radiation** can induce dimer formation between two adjacent pyrimidine bases, commonly two thymines, within a nucleotide strand. During **thymine dimer** formation, the two adjacent thymines become covalently linked and, if left unrepaired, both DNA replication and transcription are stalled at this point. DNA polymerase may proceed and replicate the dimer incorrectly, potentially leading to frameshift or point mutations.



(a) Ionizing radiation



(b) Non-ionizing radiation

(a) Ionizing radiation may lead to the formation of single-stranded and double-stranded breaks in the sugar-phosphate backbone of DNA, as well as to the modification of bases (not shown). (b) Nonionizing radiation like ultraviolet light can lead to the formation of thymine dimers, which can stall replication and transcription and introduce frameshift or point mutations.

A Summary of Mutagenic Agents

Mutagenic Agents	Mode of Action	Effect on DNA	Resulting Type of Mutation
Nucleoside analogs			
2-aminopurine	Is inserted in place of A but base pairs with C	Converts AT to GC base pair	Point
5-bromouracil	Is inserted in place of T but base pairs with G	Converts AT to GC base pair	Point
Nucleotide-modifying agent			
Nitrous oxide	Deaminates C to U	Converts GC to AT base pair	Point
Intercalating agents			
Acridine orange, ethidium bromide, polycyclic aromatic hydrocarbons	Distorts double helix, creates unusual spacing between nucleotides	Introduces small deletions and insertions	Frameshift
Ionizing radiation			

A Summary of Mutagenic Agents

Mutagenic Agents	Mode of Action	Effect on DNA	Resulting Type of Mutation
X-rays, γ -rays	Forms hydroxyl radicals	Causes single- and double-strand DNA breaks	Repair mechanisms may introduce mutations
X-rays, γ -rays	Modifies bases (e.g., deaminating C to U)	Converts GC to AT base pair	Point
Nonionizing radiation			
Ultraviolet	Forms pyrimidine (usually thymine) dimers	Causes DNA replication errors	Frameshift or point

Note:

- How does a base analog introduce a mutation?
- How does an intercalating agent introduce a mutation?
- What type of mutagen causes thymine dimers?

DNA Repair

The process of DNA replication is highly accurate, but mistakes can occur spontaneously or be induced by mutagens. Uncorrected mistakes can lead to serious consequences for the phenotype. Cells have developed several repair mechanisms to minimize the number of mutations that persist.

Proofreading

Most of the mistakes introduced during DNA replication are promptly corrected by most DNA polymerases through a function called proofreading. In proofreading, the DNA polymerase reads the newly added base, ensuring that it is complementary to the corresponding base in the template strand before adding the next one. If an incorrect base has been added, the enzyme makes a cut to release the wrong nucleotide and a new base is added.

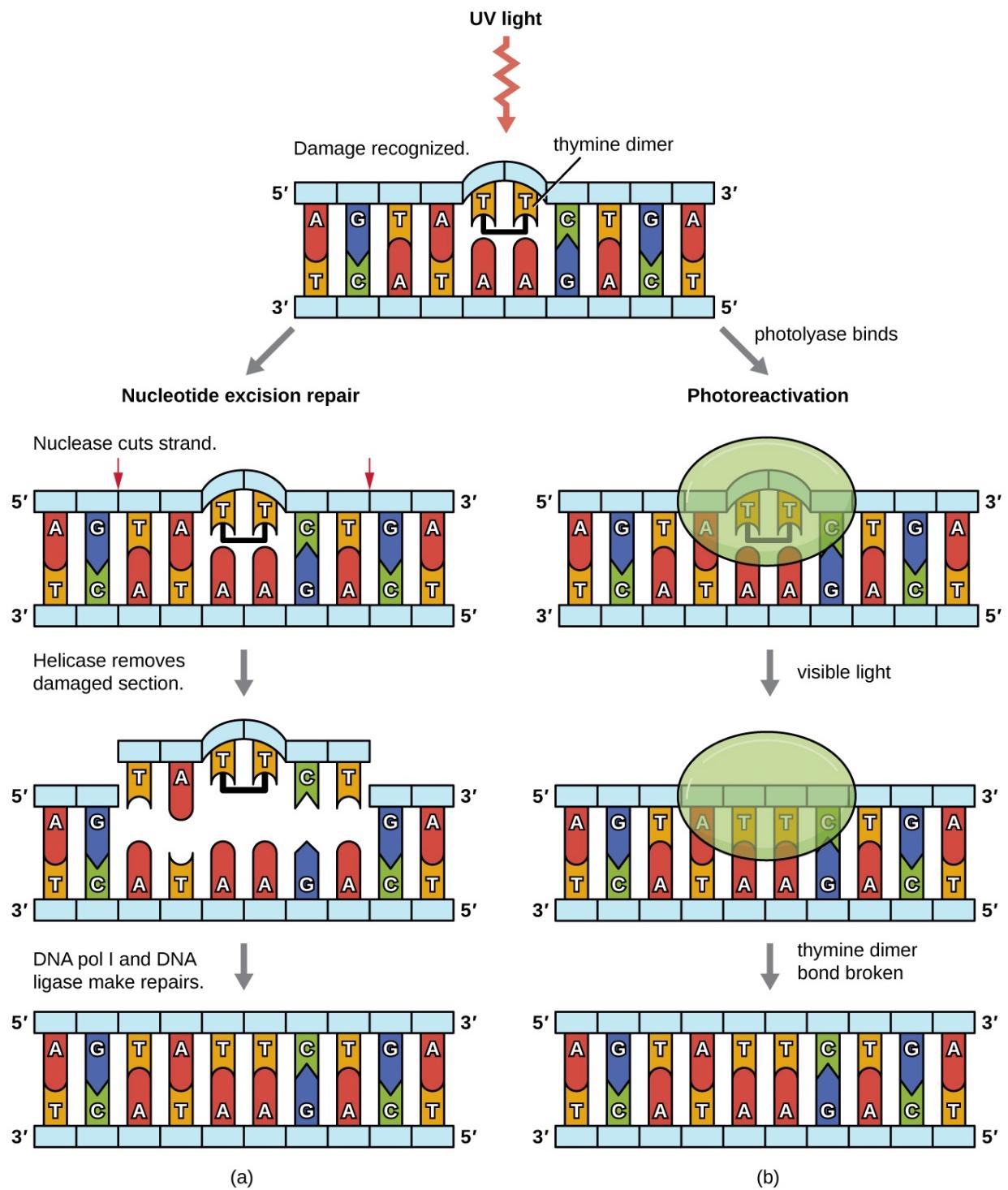
Mismatch Repair

Some errors introduced during replication are corrected shortly after the replication machinery has moved. This mechanism is called mismatch repair. The enzymes involved in this mechanism recognize the incorrectly added nucleotide, excise it, and replace it with the correct base. One example is the methyl-directed mismatch repair in *E. coli*. The DNA is hemimethylated. This means that the parental strand is methylated while the newly synthesized daughter strand is not. It takes several minutes before the new strand is methylated. Proteins MutS, MutL, and MutH bind to the hemimethylated site where the incorrect nucleotide is found. MutH cuts the nonmethylated strand (the new strand). An exonuclease removes a portion of the strand (including the incorrect nucleotide). The gap formed is then filled in by DNA pol III and ligase.

Repair of Thymine Dimers

Because the production of thymine dimers is common (many organisms cannot avoid ultraviolet light), mechanisms have evolved to repair these lesions. In **nucleotide excision repair** (also called dark repair), enzymes remove the pyrimidine dimer and replace it with the correct nucleotides ([\[link\]](#)). In *E. coli*, the DNA is scanned by an enzyme complex. If a distortion in the double helix is found that was introduced by the pyrimidine dimer, the enzyme complex cuts the sugar-phosphate backbone several bases upstream and downstream of the dimer, and the segment of DNA between these two cuts is then enzymatically removed. DNA pol I replaces the missing nucleotides with the correct ones and DNA ligase seals the gap in the sugar-phosphate backbone.

The **direct repair** (also called light repair) of thymine dimers occurs through the process of **photoreactivation** in the presence of visible light. An enzyme called photolyase recognizes the distortion in the DNA helix caused by the thymine dimer and binds to the dimer. Then, in the presence of visible light, the photolyase enzyme changes conformation and breaks apart the thymine dimer, allowing the thymines to again correctly base pair with the adenines on the complementary strand. Photoreactivation appears to be present in all organisms, with the exception of placental mammals, including humans. Photoreactivation is particularly important for organisms chronically exposed to ultraviolet radiation, like plants, photosynthetic bacteria, algae, and corals, to prevent the accumulation of mutations caused by thymine dimer formation.



Bacteria have two mechanisms for repairing thymine dimers. (a) In nucleotide excision repair, an enzyme complex recognizes the distortion in the DNA complex around the thymine dimer and cuts and removes the damaged DNA strand. The correct nucleotides are

replaced by DNA pol I and the nucleotide strand is sealed by DNA ligase. (b) In photoreactivation, the enzyme photolyase binds to the thymine dimer and, in the presence of visible light, breaks apart the dimer, restoring the base pairing of the thymines with complementary adenines on the opposite DNA strand.

Note:

- During mismatch repair, how does the enzyme recognize which is the new and which is the old strand?
- How does an intercalating agent introduce a mutation?
- What type of mutation does photolyase repair?

Identifying Bacterial Mutants

One common technique used to identify bacterial mutants is called **replica plating**. This technique is used to detect nutritional mutants, called **auxotrophs**, which have a mutation in a gene encoding an enzyme in the biosynthesis pathway of a specific nutrient, such as an amino acid. As a result, whereas wild-type cells retain the ability to grow normally on a medium lacking the specific nutrient, auxotrophs are unable to grow on such a medium. During replica plating ([\[link\]](#)), a population of bacterial cells is mutagenized and then plated as individual cells on a complex nutritionally complete plate and allowed to grow into colonies. Cells from these colonies are removed from this master plate, often using sterile velvet. This velvet, containing cells, is then pressed in the same orientation onto plates of various media. At least one plate should also be nutritionally complete to ensure that cells are being properly transferred between the plates. The other plates lack specific nutrients, allowing the researcher to discover various auxotrophic mutants unable to produce specific nutrients.

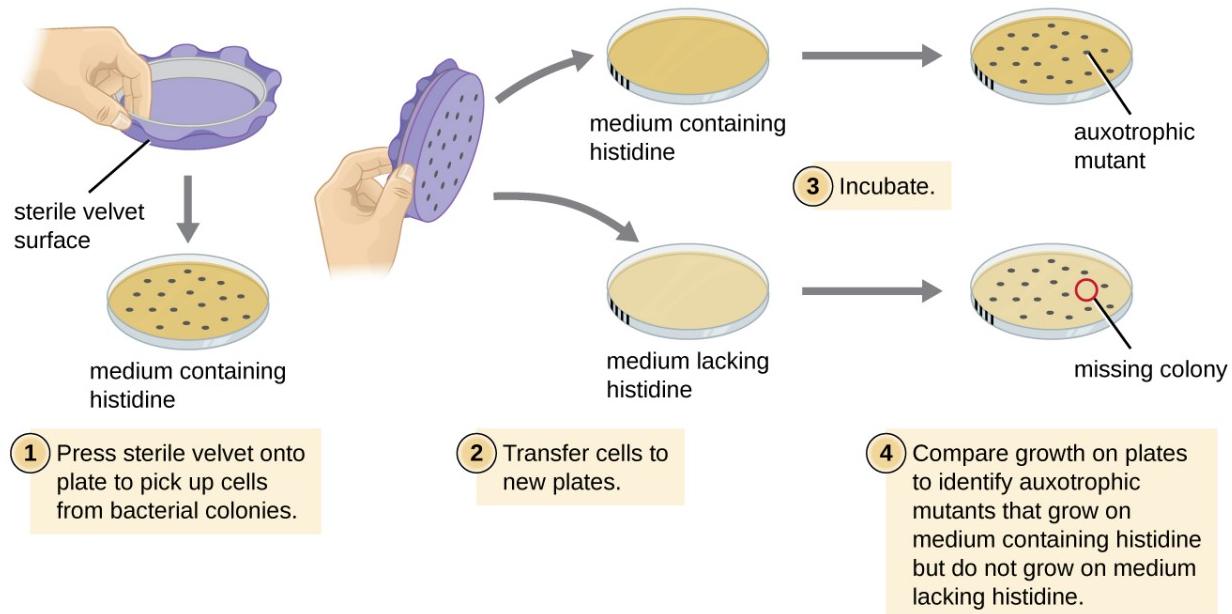
Cells from the corresponding colony on the nutritionally complete plate can be used to recover the mutant for further study.

Note:

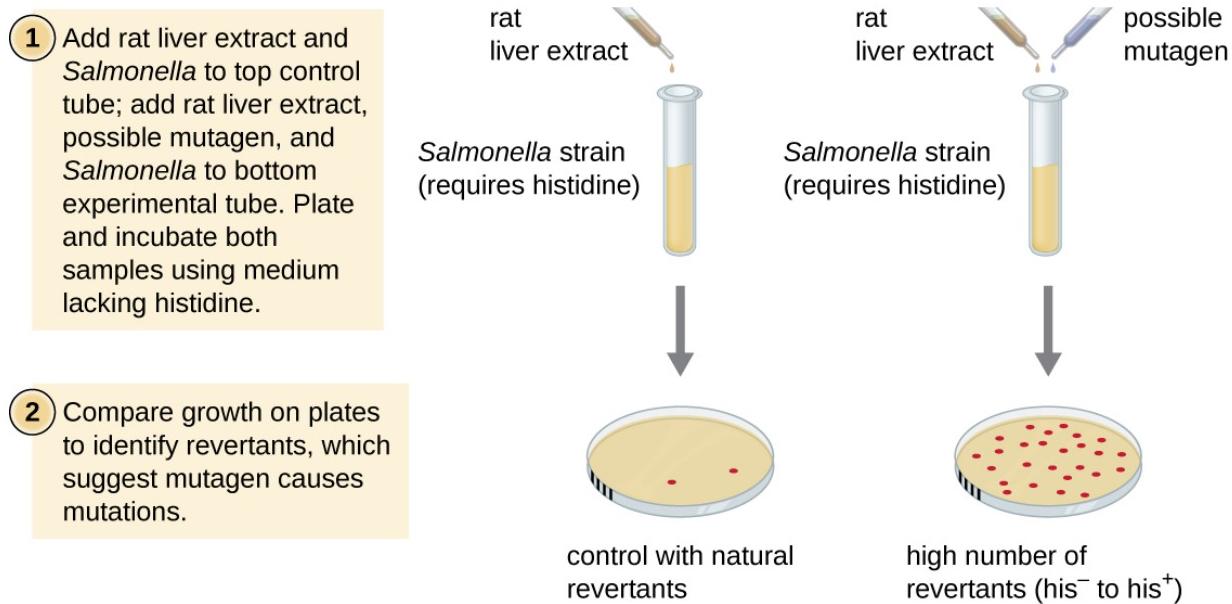
- Why are cells plated on a nutritionally complete plate in addition to nutrient-deficient plates when looking for a mutant?

The Ames Test

The **Ames test**, developed by Bruce Ames (1928–) in the 1970s, is a method that uses bacteria for rapid, inexpensive screening of the carcinogenic potential of new chemical compounds. The test measures the mutation rate associated with exposure to the compound, which, if elevated, may indicate that exposure to this compound is associated with greater cancer risk. The Ames test uses as the test organism a strain of *Salmonella typhimurium* that is a histidine auxotroph, unable to synthesize its own histidine because of a mutation in an essential gene required for its synthesis. After exposure to a potential mutagen, these bacteria are plated onto a medium lacking histidine, and the number of mutants regaining the ability to synthesize histidine is recorded and compared with the number of such mutants that arise in the absence of the potential mutagen ([\[link\]](#)). Chemicals that are more mutagenic will bring about more mutants with restored histidine synthesis in the Ames test. Because many chemicals are not directly mutagenic but are metabolized to mutagenic forms by liver enzymes, rat liver extract is commonly included at the start of this experiment to mimic liver metabolism. After the Ames test is conducted, compounds identified as mutagenic are further tested for their potential carcinogenic properties by using other models, including animal models like mice and rats.



Identification of auxotrophic mutants, like histidine auxotrophs, is done using replica plating. After mutagenesis, colonies that grow on nutritionally complete medium but not on medium lacking histidine are identified as histidine auxotrophs.



The Ames test is used to identify mutagenic, potentially carcinogenic chemicals. A *Salmonella* histidine auxotroph is used as the test strain, exposed to a potential mutagen/carcinogen. The number of reversion mutants capable of growing in the absence of supplied histidine is counted and compared with the number of natural reversion mutants that arise in the absence of the potential mutagen.

Note:

- What mutation is used as an indicator of mutation rate in the Ames test?
- Why can the Ames test work as a test for carcinogenicity?

Key Concepts and Summary

- A **mutation** is a heritable change in DNA. A mutation may lead to a change in the amino-acid sequence of a protein, possibly affecting its function.
- A **point mutation** affects a single base pair. A point mutation may cause a **silent mutation** if the mRNA codon codes for the same amino acid, a **missense mutation** if the mRNA codon codes for a different amino acid, or a **nonsense mutation** if the mRNA codon becomes a stop codon.
- Missense mutations may retain function, depending on the chemistry of the new amino acid and its location in the protein. Nonsense mutations produce truncated and frequently nonfunctional proteins.
- A **frameshift mutation** results from an insertion or deletion of a number of nucleotides that is not a multiple of three. The change in reading frame alters every amino acid after the point of the mutation and results in a nonfunctional protein.

- **Spontaneous mutations** occur through DNA replication errors, whereas **induced mutations** occur through exposure to a **mutagen**.
- Mutagenic agents are frequently carcinogenic but not always. However, nearly all carcinogens are mutagenic.
- Chemical mutagens include base analogs and chemicals that modify existing bases. In both cases, mutations are introduced after several rounds of DNA replication.
- **Ionizing radiation**, such as X-rays and γ -rays, leads to breakage of the phosphodiester backbone of DNA and can also chemically modify bases to alter their base-pairing rules.
- **Nonionizing radiation** like ultraviolet light may introduce pyrimidine (thymine) dimers, which, during DNA replication and transcription, may introduce frameshift or point mutations.
- Cells have mechanisms to repair naturally occurring mutations. DNA polymerase has proofreading activity. Mismatch repair is a process to repair incorrectly incorporated bases after DNA replication has been completed.
- Pyrimidine dimers can also be repaired. In **nucleotide excision repair (dark repair)**, enzymes recognize the distortion introduced by the pyrimidine dimer and replace the damaged strand with the correct bases, using the undamaged DNA strand as a template. Bacteria and other organisms may also use **direct repair**, in which the photolyase enzyme, in the presence of visible light, breaks apart the pyrimidines.
- Through comparison of growth on the complete plate and lack of growth on media lacking specific nutrients, specific loss-of-function mutants called **auxotrophs** can be identified.
- The **Ames test** is an inexpensive method that uses auxotrophic bacteria to measure mutagenicity of a chemical compound. Mutagenicity is an indicator of carcinogenic potential.

Short Answer

Exercise:

Problem:

Why is it more likely that insertions or deletions will be more detrimental to a cell than point mutations?

Critical Thinking

Exercise:

Problem:

Below are several DNA sequences that are mutated compared with the wild-type sequence: 3'-T A C T G A C T G A C G A T C-5'. Envision that each is a section of a DNA molecule that has separated in preparation for transcription, so you are only seeing the template strand. Construct the complementary DNA sequences (indicating 5' and 3' ends) for each mutated DNA sequence, then transcribe (indicating 5' and 3' ends) the template strands, and translate the mRNA molecules using the genetic code, recording the resulting amino acid sequence (indicating the N and C termini). What type of mutation is each?

Mutated DNA Template Strand #1: 3'-T A C T G T C T G A C G A T C-5'

Complementary DNA sequence:

mRNA sequence transcribed from template:

Amino acid sequence of peptide:

Type of mutation:

Mutated DNA Template Strand #2: 3'-T A C G G A C T G A C G A T C-5'

Complementary DNA sequence:

mRNA sequence transcribed from template:

Amino acid sequence of peptide:

Type of mutation:

Mutated DNA Template Strand #3: 3'-T A C T G A C T G A C T A T C-5'

Complementary DNA sequence:

mRNA sequence transcribed from template:

Amino acid sequence of peptide:

Type of mutation:

Mutated DNA Template Strand #4: 3'-T A C G A C T G A C T A T C-5'

Complementary DNA sequence:

mRNA sequence transcribed from template:

Amino acid sequence of peptide:

Type of mutation:

Exercise:

Problem:

Why do you think the Ames test is preferable to the use of animal models to screen chemical compounds for mutagenicity?

How Asexual Prokaryotes Achieve Genetic Diversity

LEARNING OBJECTIVES

- Compare the processes of transformation, transduction, and conjugation
- Explain how asexual gene transfer results in prokaryotic genetic diversity
- Explain the structure and consequences for bacterial genetic diversity of transposons

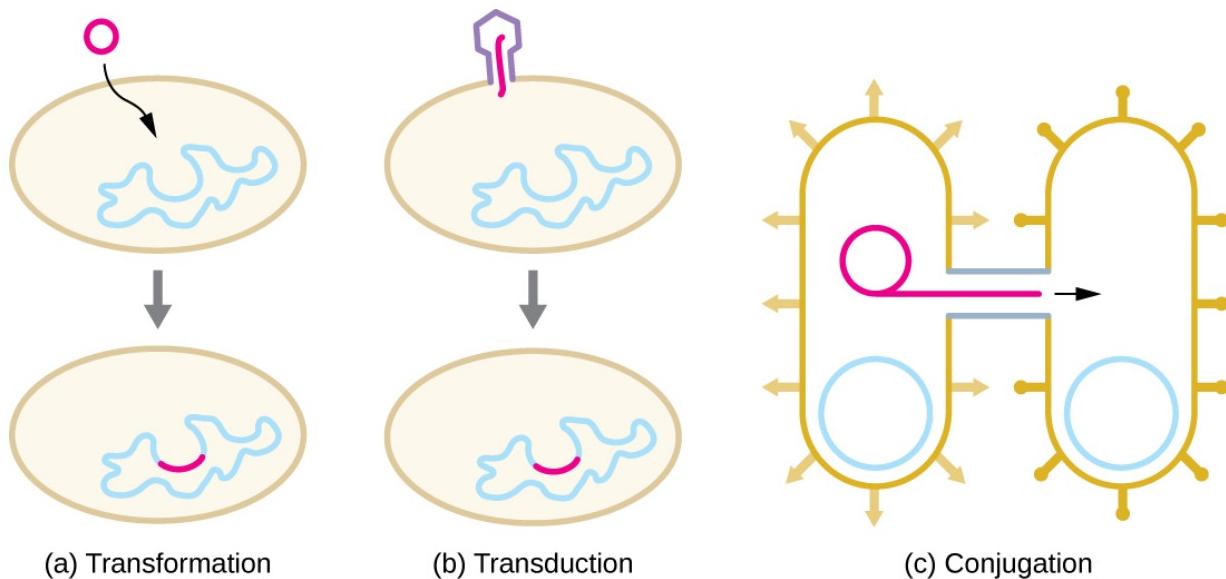
Typically, when we consider genetic transfer, we think of **vertical gene transfer**, the transmission of genetic information from generation to generation. Vertical gene transfer is by far the main mode of transmission of genetic information in all cells. In sexually reproducing organisms, crossing-over events and independent assortment of individual chromosomes during meiosis contribute to genetic diversity in the population. Genetic diversity is also introduced during sexual reproduction, when the genetic information from two parents, each with different complements of genetic information, are combined, producing new combinations of parental genotypes in the diploid offspring. The occurrence of mutations also contributes to genetic diversity in a population. Genetic diversity of offspring is useful in changing or inconsistent environments and may be one reason for the evolutionary success of sexual reproduction.

When prokaryotes and eukaryotes reproduce asexually, they transfer a nearly identical copy of their genetic material to their offspring through vertical gene transfer. Although asexual reproduction produces more offspring more quickly, any benefits of diversity among those offspring are

lost. How then do organisms whose dominant reproductive mode is asexual create genetic diversity? In prokaryotes, **horizontal gene transfer (HGT)**, the introduction of genetic material from one organism to another organism within the same generation, is an important way to introduce genetic diversity. HGT allows even distantly related species to share genes, influencing their phenotypes. It is thought that HGT is more prevalent in prokaryotes but that only a small fraction of the prokaryotic genome may be transferred by this type of transfer at any one time. As the phenomenon is investigated more thoroughly, it may be revealed to be even more common. Many scientists believe that HGT and mutation are significant sources of genetic variation, the raw material for the process of natural selection, in prokaryotes. Although HGT is more common among evolutionarily related organisms, it may occur between any two species that live together in a natural community.

HGT in prokaryotes is known to occur by the three primary mechanisms that are illustrated in [\[link\]](#):

1. Transformation: naked DNA is taken up from the environment
2. Transduction: genes are transferred between cells in a virus (see [The Viral Life Cycle](#))
3. Conjugation: use of a hollow tube called a conjugation pilus to transfer genes between cells



There are three prokaryote-specific mechanisms leading to horizontal gene transfer in prokaryotes. a) In transformation, the cell takes up DNA directly from the environment. The DNA may remain separate as a plasmid or be incorporated into the host genome. b) In transduction, a bacteriophage injects DNA that is a hybrid of viral DNA and DNA from a previously infected bacterial cell. c) In conjugation, DNA is transferred between cells through a cytoplasmic bridge after a conjugation pilus draws the two cells close enough to form the bridge.

Note:

- What are three ways sexual reproduction introduces genetic variation into offspring?
- What is a benefit of asexual reproduction?
- What are the three mechanisms of horizontal gene transfer in prokaryotes?

Transformation

Frederick Griffith was the first to demonstrate the process of transformation. In 1928, he showed that live, nonpathogenic *Streptococcus pneumoniae* bacteria could be transformed into pathogenic bacteria through exposure to a heat-killed pathogenic strain. He concluded that some sort of agent, which he called the “transforming principle,” had been passed from the dead pathogenic bacteria to the live, nonpathogenic bacteria. In 1944, Oswald Avery (1877–1955), Colin MacLeod (1909–1972), and Maclyn McCarty (1911–2005) demonstrated that the transforming principle was DNA (see [Using Microorganisms to Discover the Secrets of Life](#)).

In **transformation**, the prokaryote takes up naked DNA found in its environment and that is derived from other cells that have lysed on death and released their contents, including their genome, into the environment. Many bacteria are naturally competent, meaning that they actively bind to environmental DNA, transport it across their cell envelopes into their cytoplasm, and make it single stranded. Typically, double-stranded foreign DNA within cells is destroyed by nucleases as a defense against viral infection. However, these nucleases are usually ineffective against single-stranded DNA, so this single-stranded DNA within the cell has the opportunity to recombine into the bacterial genome. A molecule of DNA that contains fragments of DNA from different organisms is called recombinant DNA. (Recombinant DNA will be discussed in more detail in [Microbes and the Tools of Genetic Engineering](#).) If the bacterium incorporates the new DNA into its own genome through recombination, the bacterial cell may gain new phenotypic properties. For example, if a nonpathogenic bacterium takes up DNA for a toxin gene from a pathogen and then incorporates it into its chromosome, it, too, may become pathogenic. Plasmid DNA may also be taken up by competent bacteria and confer new properties to the cell. Overall, transformation in nature is a relatively inefficient process because environmental DNA levels are low because of the activity of nucleases that are also released during cellular lysis. Additionally, genetic recombination is inefficient at incorporating new DNA sequences into the genome.

In nature, bacterial transformation is an important mechanism for the acquisition of genetic elements encoding virulence factors and antibiotic resistance. Genes encoding resistance to antimicrobial compounds have been shown to be widespread in nature, even in environments not influenced by humans. These genes, which allow microbes living in mixed communities to compete for limited resources, can be transferred within a population by transformation, as well as by the other processes of HGT. In the laboratory, we can exploit the natural process of bacterial transformation for genetic engineering to make a wide variety of medicinal products, as discussed in [Microbes and the Tools of Genetic Engineering](#).

Note:

- Why does a bacterial cell make environmental DNA brought into the cell into a single-stranded form?

Transduction

Viruses that infect bacteria (bacteriophages) may also move short pieces of chromosomal DNA from one bacterium to another in a process called **transduction** (see [\[link\]](#)). Recall that in generalized transduction, any piece of chromosomal DNA may be transferred to a new host cell by accidental packaging of chromosomal DNA into a phage head during phage assembly. By contrast, specialized transduction results from the imprecise excision of a lysogenic prophage from the bacterial chromosome such that it carries with it a piece of the bacterial chromosome from either side of the phage's integration site to a new host cell. As a result, the host may acquire new properties. This process is called lysogenic conversion. Of medical significance, a lysogenic phage may carry with it a virulence gene to its new host. Once inserted into the new host's chromosome, the new host may gain pathogenicity. Several pathogenic bacteria, including *Corynebacterium diphtheriae* (the causative agent of diphtheria) and *Clostridium botulinum* (the causative agent of botulism), are virulent because of the introduction of toxin-encoding genes by lysogenic bacteriophages, affirming the clinical relevance of transduction in the exchange of genes involved in infectious disease. Archaea have their own viruses that translocate genetic material from one individual to another.

Note:

- What is the agent of transduction of prokaryotic cells?
- In specialized transduction, where does the transducing piece of DNA come from?

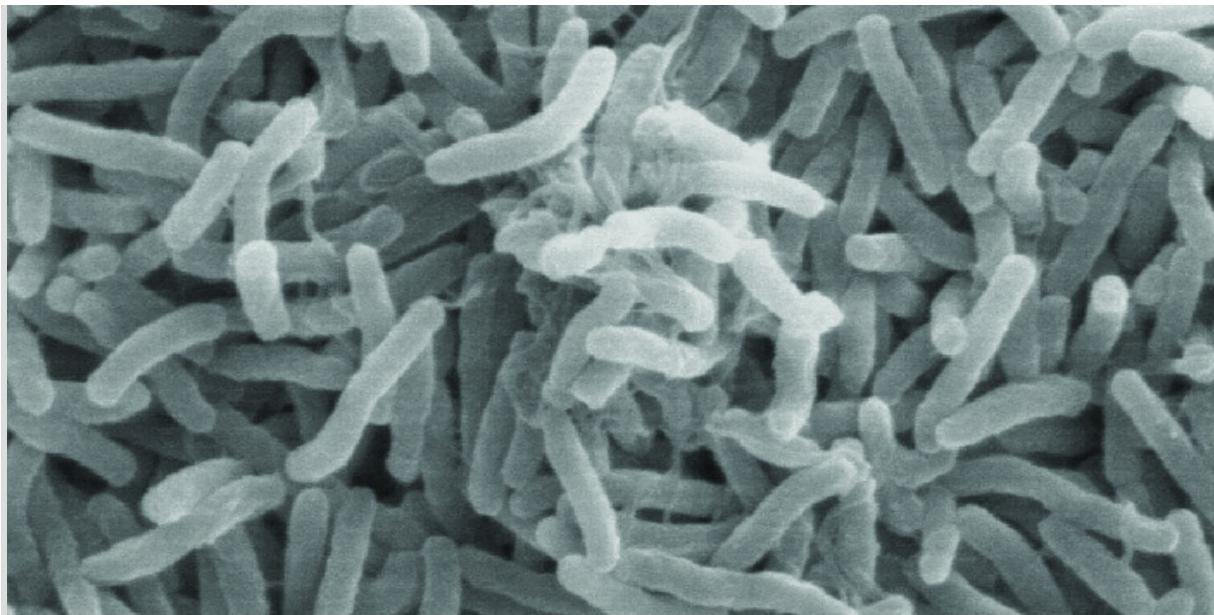
Note:

The Clinical Consequences of Transduction

Paul, a 23-year-old relief worker from Atlanta, traveled to Haiti in 2011 to provide aid following the 2010 earthquake. After working there for several weeks, he suddenly began experiencing abdominal distress, including severe cramping, nausea, vomiting, and watery diarrhea. He also began to experience intense muscle cramping. At a local clinic, the physician suspected that Paul's symptoms were caused by cholera because there had been a cholera outbreak after the earthquake. Because cholera is transmitted by the fecal-oral route, breaches in sanitation infrastructure, such as often occur following natural disasters, may precipitate outbreaks. The physician confirmed the presumptive diagnosis using a cholera dipstick test. He then prescribed Paul a single dose of doxycycline, as well as oral rehydration salts, instructing him to drink significant amounts of clean water.

Cholera is caused by the gram-negative curved rod *Vibrio cholerae* ([\[link\]](#)). Its symptoms largely result from the production of the cholera toxin (CT), which ultimately activates a chloride transporter to pump chloride ions out of the epithelial cells into the gut lumen. Water then follows the chloride ions, causing the prolific watery diarrhea characteristic of cholera. The gene encoding the cholera toxin is incorporated into the bacterial chromosome of *V. cholerae* through infection of the bacterium with the lysogenic filamentous CTX phage, which carries the CT gene and introduces it into the chromosome on integration of the prophage. Thus, pathogenic strains of *V. cholerae* result from horizontal gene transfer by specialized transduction.

- Why are outbreaks of cholera more common as a result of a natural disaster?
- Why is muscle cramping a common symptom of cholera? Why is treatment with oral rehydration salts so important for the treatment of cholera?
- In areas stricken by cholera, what are some strategies that people could use to prevent disease transmission?



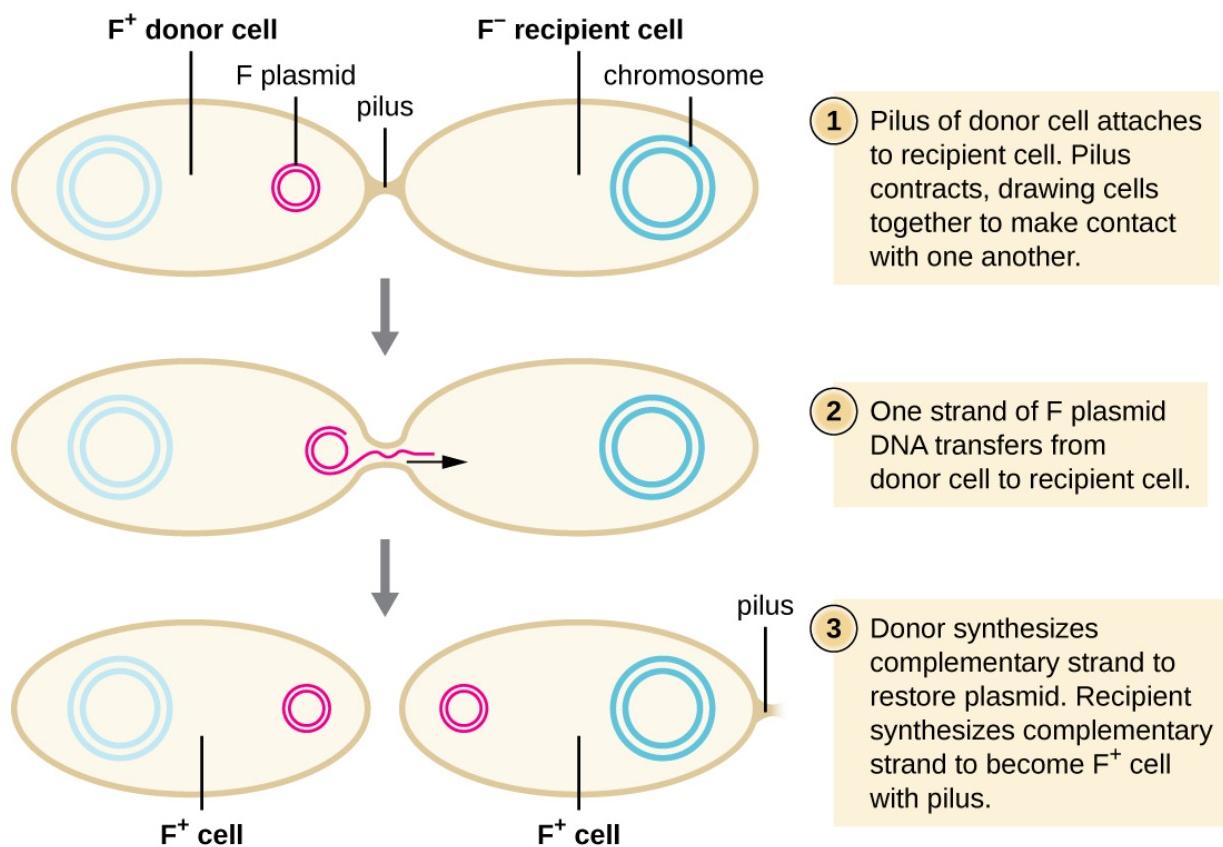
A scanning electron micrograph of *Vibrio cholerae* shows its characteristic curved rod shape.

Conjugation

In **conjugation**, DNA is directly transferred from one prokaryote to another by means of a **conjugation pilus**, which brings the organisms into contact with one another. In *E. coli*, the genes encoding the ability to conjugate are located on a bacterial plasmid called the **F plasmid**, also known as the **fertility factor**, and the conjugation pilus is called the **F pilus**. The F- plasmid genes encode both the proteins composing the F pilus and those involved in rolling circle replication of the plasmid. Cells containing the F plasmid, capable of forming an F pilus, are called **F⁺ cells** or **donor cells**, and those lacking an F plasmid are called **F⁻ cells** or **recipient cells**.

Conjugation of the F Plasmid

During typical conjugation in *E. coli*, the F pilus of an F⁺ cell comes into contact with an F⁻ cell and retracts, bringing the two cell envelopes into contact ([\[link\]](#)). Then a cytoplasmic bridge forms between the two cells at the site of the conjugation pilus. As rolling circle replication of the F plasmid occurs in the F⁺ cell, a single-stranded copy of the F plasmid is transferred through the cytoplasmic bridge to the F⁻ cell, which then synthesizes the complementary strand, making it double stranded. The F⁻ cell now becomes an F⁺ cell capable of making its own conjugation pilus. Eventually, in a mixed bacterial population containing both F⁺ and F⁻ cells, all cells will become F⁺ cells. Genes on the *E. coli* F plasmid also encode proteins preventing conjugation between F⁺ cells.

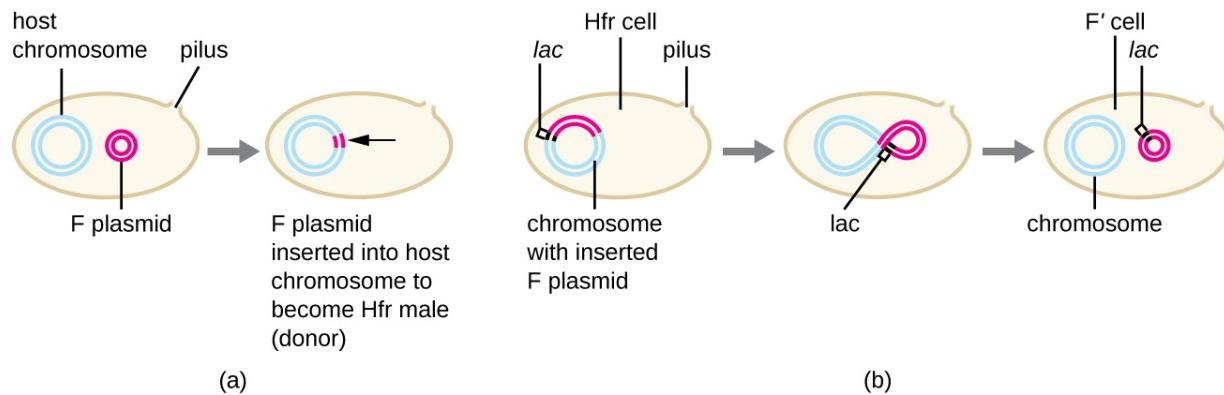


Typical conjugation of the F plasmid from an F⁺ cell to an F⁻ cell is brought about by the conjugation pilus bringing the two cells into contact. A single strand of the F plasmid is transferred to the F⁻ cell, which is then made double stranded.

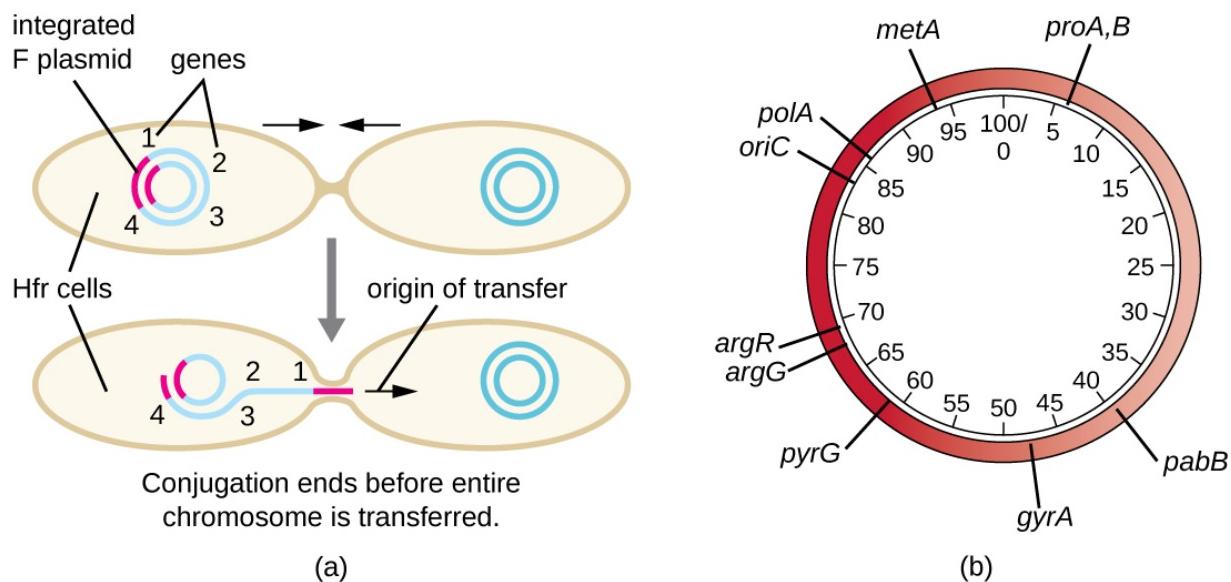
Conjugation of F' and Hfr Cells

Although typical conjugation in *E. coli* results in the transfer of the F-plasmid DNA only, conjugation may also transfer chromosomal DNA. This is because the F plasmid occasionally integrates into the bacterial chromosome through recombination between the plasmid and the chromosome, forming an **Hfr cell** ([\[link\]](#)). “Hfr” refers to the high frequency of recombination seen when recipient F⁻ cells receive genetic information from Hfr cells through conjugation. Similar to the imprecise excision of a prophage during specialized transduction, the integrated F plasmid may also be imprecisely excised from the chromosome, producing an **F' plasmid** that carries with it some chromosomal DNA adjacent to the integration site. On conjugation, this DNA is introduced to the recipient cell and may be either maintained as part of the F' plasmid or be recombined into the recipient cell's bacterial chromosome.

Hfr cells may also treat the bacterial chromosome like an enormous F plasmid and attempt to transfer a copy of it to a recipient F⁻ cell. Because the bacterial chromosome is so large, transfer of the entire chromosome takes a long time ([\[link\]](#)). However, contact between bacterial cells during conjugation is transient, so it is unusual for the entire chromosome to be transferred. Host chromosomal DNA near the integration site of the F plasmid, displaced by the unidirectional process of rolling circle replication, is more likely to be transferred and recombined into a recipient cell's chromosome than host genes farther away. Thus, the relative location of bacterial genes on the Hfr cell's genome can be mapped based on when they are transferred through conjugation. As a result, prior to the age of widespread bacterial genome sequencing, distances on prokaryotic genome maps were often measured in minutes.



(a) The *F* plasmid can occasionally integrate into the bacterial chromosome, producing an *Hfr* cell. (b) Imprecise excision of the *F* plasmid from the chromosome of an *Hfr* cell may lead to the production of an *F'* plasmid that carries chromosomal DNA adjacent to the integration site. This *F'* plasmid can be transferred to an *F⁻* cell by conjugation.



(a) An *Hfr* cell may attempt to transfer the entire bacterial chromosome to an *F⁻* cell, treating the chromosome like an extremely large *F* plasmid. However, contact between cells during conjugation is

temporary. Chromosomal genes closest to the integration site (gene 1) that are first displaced during rolling circle replication will be transferred more quickly than genes far away from the integration site (gene 4). Hence, they are more likely to be recombined into the recipient F⁻ cell's chromosome. (b) The time it takes for a gene to be transferred, as detected by recombination into the F⁻ cell's chromosome, can be used to generate a map of the bacterial genome, such as this genomic map of *E. coli*. Note that it takes approximately 100 minutes for the entire genome (4.6 Mbp) of an Hfr strain of *E. coli* to be transferred by conjugation.

Consequences and Applications of Conjugation

Plasmids are an important type of extrachromosomal DNA element in bacteria and, in those cells that harbor them, are considered to be part of the bacterial genome. From a clinical perspective, plasmids often code for genes involved in virulence. For example, genes encoding proteins that make a bacterial cell resistant to a particular antibiotic are encoded on **R plasmids**. R plasmids, in addition to their genes for antimicrobial resistance, contain genes that control conjugation and transfer of the plasmid. R plasmids are able to transfer between cells of the same species and between cells of different species. Single R plasmids commonly contain multiple genes conferring resistance to multiple antibiotics.

Genes required for the production of various toxins and molecules important for colonization during infection may also be found encoded on plasmids. For example, verotoxin-producing strains of *E. coli* (VTEC) appear to have acquired the genes encoding the Shiga toxin from its gram-negative relative *Shigella dysenteriae* through the acquisition of a large plasmid encoding this toxin. VTEC causes severe diarrheal disease that may result in hemolytic uremic syndrome (HUS), which may lead to kidney failure and death.

In nonclinical settings, bacterial genes that encode metabolic enzymes needed to degrade specialized atypical compounds like polycyclic aromatic hydrocarbons (PAHs) are also frequently encoded on plasmids. Additionally, certain plasmids have the ability to move from bacterial cells to other cell types, like those of plants and animals, through mechanisms distinct from conjugation. Such mechanisms and their use in genetic engineering are covered in [Modern Applications of Microbial Genetics](#).

Note:



Click through this [animation](#) to learn more about the process of conjugation.

Note:

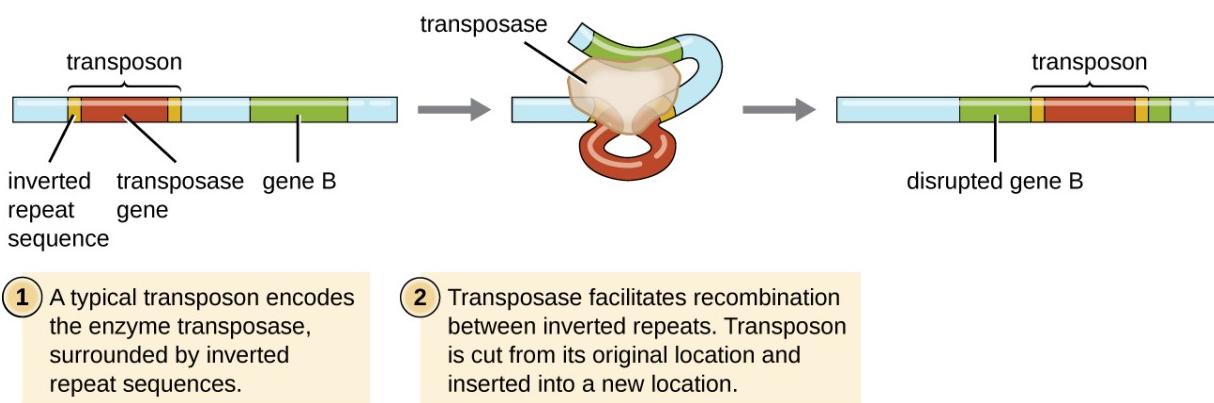
- What type of replication occurs during conjugation?
- What occurs to produce an Hfr *E. coli* cell?
- What types of traits are encoded on plasmids?

Transposition

Genetic elements called **transposons** (transposable elements), or “jumping genes,” are molecules of DNA that include special inverted repeat

sequences at their ends and a gene encoding the enzyme transposase ([\[link\]](#)). Transposons allow the entire sequence to independently excise from one location in a DNA molecule and integrate into the DNA elsewhere through a process called **transposition**. Transposons were originally discovered in maize (corn) by American geneticist Barbara McClintock (1902–1992) in the 1940s. Transposons have since been found in all types of organisms, both prokaryotes and eukaryotes. Thus, unlike the three previous mechanisms discussed, transposition is not prokaryote-specific. Most transposons are nonreplicative, meaning they move in a “cut-and-paste” fashion. Some may be replicative, however, retaining their location in the DNA while making a copy to be inserted elsewhere (“copy and paste”). Because transposons can move within a DNA molecule, from one DNA molecule to another, or even from one cell to another, they have the ability to introduce genetic diversity. Movement within the same DNA molecule can alter phenotype by inactivating or activating a gene.

Transposons may carry with them additional genes, moving these genes from one location to another with them. For example, bacterial transposons can relocate antibiotic resistance genes, moving them from chromosomes to plasmids. This mechanism has been shown to be responsible for the colocalization of multiple antibiotic resistance genes on a single R plasmid in *Shigella* strains causing bacterial dysentery. Such an R plasmid can then be easily transferred among a bacterial population through the process of conjugation.



Transposons are segments of DNA that have the ability to move from

one location to another because they code for the enzyme transposase. In this example, a nonreplicative transposon has disrupted gene B. The consequence of that the transcription of gene B may now have been interrupted.

Note:

- What are two ways a transposon can affect the phenotype of a cell it moves to?

[[link](#)] summarizes the processes discussed in this section.

Summary of Mechanisms of Genetic Diversity in Prokaryotes

Term	Definition
Conjugation	Transfer of DNA through direct contact using a conjugation pilus
Transduction	Mechanism of horizontal gene transfer in bacteria in which genes are transferred through viral infection

Summary of Mechanisms of Genetic Diversity in Prokaryotes

Term	Definition
Transformation	Mechanism of horizontal gene transfer in which naked environmental DNA is taken up by a bacterial cell
Transposition	Process whereby DNA independently excises from one location in a DNA molecule and integrates elsewhere

Key Concepts and Summary

- **Horizontal gene transfer** is an important way for asexually reproducing organisms like prokaryotes to acquire new traits.
- There are three mechanisms of horizontal gene transfer typically used by bacteria: **transformation**, **transduction**, and **conjugation**.
- Transformation allows for competent cells to take up naked DNA, released from other cells on their death, into their cytoplasm, where it may recombine with the host genome.
- In **generalized transduction**, any piece of chromosomal DNA may be transferred by accidental packaging of the degraded host chromosome into a phage head. In **specialized transduction**, only chromosomal DNA adjacent to the integration site of a lysogenic phage may be transferred as a result of imprecise excision of the prophage.
- Conjugation is mediated by the **F plasmid**, which encodes a **conjugation pilus** that brings an F plasmid-containing **F⁺ cell** into contact with an **F⁻ cell**.
- The rare integration of the F plasmid into the bacterial chromosome, generating an **Hfr cell**, allows for transfer of chromosomal DNA from the donor to the recipient. Additionally, imprecise excision of the F plasmid from the chromosome may generate an F' plasmid that may be transferred to a recipient by conjugation.
- Conjugation transfer of **R plasmids** is an important mechanism for the spread of antibiotic resistance in bacterial communities.

- **Transposons** are molecules of DNA with inverted repeats at their ends that also encode the enzyme transposase, allowing for their movement from one location in DNA to another. Although found in both prokaryotes and eukaryotes, transposons are clinically relevant in bacterial pathogens for the movement of virulence factors, including antibiotic resistance genes.

Short Answer

Exercise:

Problem:

Briefly describe two ways in which chromosomal DNA from a donor cell may be transferred to a recipient cell during the process of conjugation.

Exercise:

Problem:

Describe what happens when a nonsense mutation is introduced into the gene encoding transposase within a transposon.

Gene Regulation: Operon Theory

LEARNING OBJECTIVES

- Compare inducible operons and repressible operons
- Describe why regulation of operons is important

Each nucleated cell in a multicellular organism contains copies of the same DNA. Similarly, all cells in two pure bacterial cultures inoculated from the same starting colony contain the same DNA, with the exception of changes that arise from spontaneous mutations. If each cell in a multicellular organism has the same DNA, then how is it that cells in different parts of the organism's body exhibit different characteristics? Similarly, how is it that the same bacterial cells within two pure cultures exposed to different environmental conditions can exhibit different phenotypes? In both cases, each genetically identical cell does not turn on, or express, the same set of genes. Only a subset of proteins in a cell at a given time is expressed.

Genomic DNA contains both structural genes, which encode products that serve as cellular structures or enzymes, and regulatory genes, which encode products that regulate gene expression. The expression of a gene is a highly regulated process. Whereas regulating gene expression in multicellular organisms allows for cellular differentiation, in single-celled organisms like prokaryotes, it primarily ensures that a cell's resources are not wasted making proteins that the cell does not need at that time.

Elucidating the mechanisms controlling gene expression is important to the understanding of human health. Malfunctions in this process in humans lead to the development of cancer and other diseases. Understanding the

interaction between the gene expression of a pathogen and that of its human host is important for the understanding of a particular infectious disease. Gene regulation involves a complex web of interactions within a given cell among signals from the cell's environment, signaling molecules within the cell, and the cell's DNA. These interactions lead to the expression of some genes and the suppression of others, depending on circumstances.

Prokaryotes and eukaryotes share some similarities in their mechanisms to regulate gene expression; however, gene expression in eukaryotes is more complicated because of the temporal and spatial separation between the processes of transcription and translation. Thus, although most regulation of gene expression occurs through transcriptional control in prokaryotes, regulation of gene expression in eukaryotes occurs at the transcriptional level and post-transcriptionally (after the primary transcript has been made).

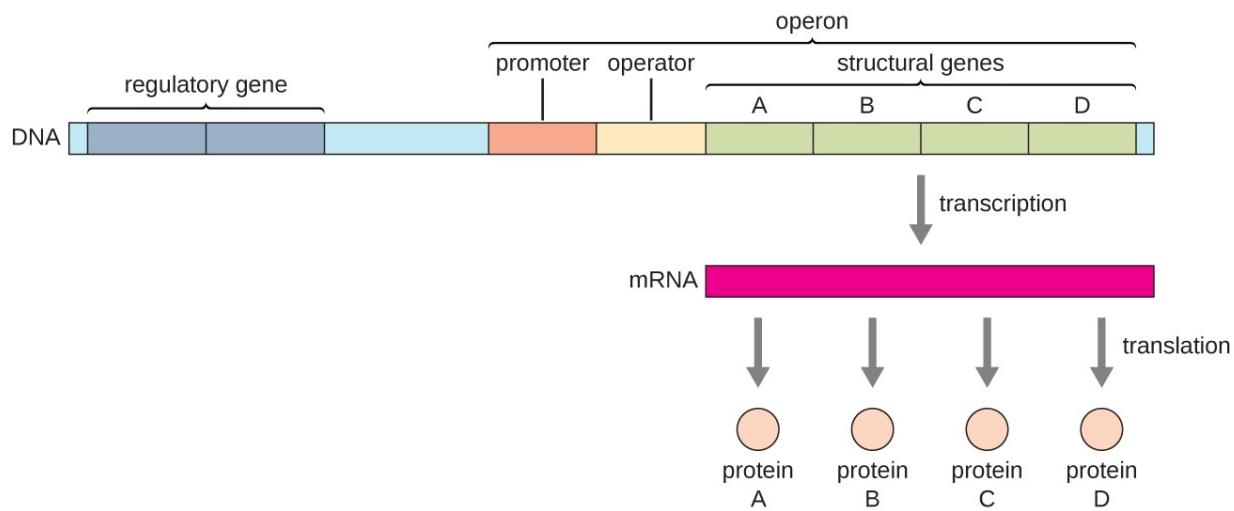
Prokaryotic Gene Regulation

In bacteria and archaea, structural proteins with related functions are usually encoded together within the genome in a block called an **operon** and are transcribed together under the control of a single promoter, resulting in the formation of a polycistronic transcript ([\[link\]](#)). In this way, regulation of the transcription of all of the structural genes encoding the enzymes that catalyze the many steps in a single biochemical pathway can be controlled simultaneously, because they will either all be needed at the same time, or none will be needed. For example, in *E. coli*, all of the structural genes that encode enzymes needed to use lactose as an energy source lie next to each other in the lactose (or *lac*) operon under the control of a single promoter, the *lac* promoter. French scientists François Jacob (1920–2013) and Jacques Monod at the Pasteur Institute were the first to show the organization of bacterial genes into operons, through their studies on the *lac* operon of *E. coli*. For this work, they won the Nobel Prize in Physiology or Medicine in 1965. Although eukaryotic genes are not organized into operons, prokaryotic operons are excellent models for learning about gene regulation generally. There are some gene clusters in eukaryotes that function similar to operons. Many of the principles can be applied to eukaryotic systems and contribute to our understanding of

changes in gene expression in eukaryotes that can result pathological changes such as cancer.

Each operon includes DNA sequences that influence its own transcription; these are located in a region called the regulatory region. The regulatory region includes the promoter and the region surrounding the promoter, to which **transcription factors**, proteins encoded by regulatory genes, can bind. Transcription factors influence the binding of RNA polymerase to the promoter and allow its progression to transcribe structural genes. A **repressor** is a transcription factor that suppresses transcription of a gene in response to an external stimulus by binding to a DNA sequence within the regulatory region called the **operator**, which is located between the RNA polymerase binding site of the promoter and the transcriptional start site of the first structural gene. Repressor binding physically blocks RNA polymerase from transcribing structural genes. Conversely, an **activator** is a transcription factor that increases the transcription of a gene in response to an external stimulus by facilitating RNA polymerase binding to the promoter. An **inducer**, a third type of regulatory molecule, is a small molecule that either activates or represses transcription by interacting with a repressor or an activator.

In prokaryotes, there are examples of operons whose gene products are required rather consistently and whose expression, therefore, is unregulated. Such operons are **constitutively expressed**, meaning they are transcribed and translated continuously to provide the cell with constant intermediate levels of the protein products. Such genes encode enzymes involved in housekeeping functions required for cellular maintenance, including DNA replication, repair, and expression, as well as enzymes involved in core metabolism. In contrast, there are other prokaryotic operons that are expressed only when needed and are regulated by repressors, activators, and inducers.



In prokaryotes, structural genes of related function are often organized together on the genome and transcribed together under the control of a single promoter. The operon's regulatory region includes both the promoter and the operator. If a repressor binds to the operator, then the structural genes will not be transcribed. Alternatively, activators may bind to the regulatory region, enhancing transcription.

Note:

- What are the parts in the DNA sequence of an operon?
- What types of regulatory molecules are there?

Regulation by Repression

Prokaryotic operons are commonly controlled by the binding of repressors to operator regions, thereby preventing the transcription of the structural genes. Such operons are classified as either **repressible operons** or inducible operons. Repressible operons, like the tryptophan (*trp*) operon, typically contain genes encoding enzymes required for a biosynthetic

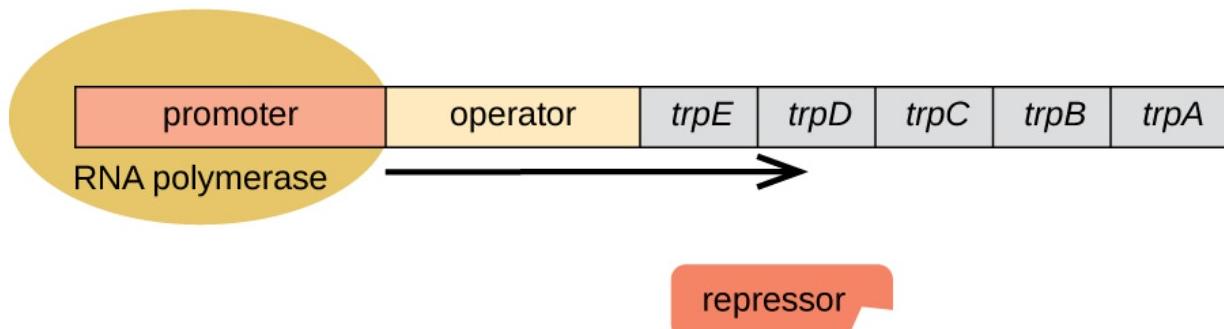
pathway. As long as the product of the pathway, like tryptophan, continues to be required by the cell, a repressible operon will continue to be expressed. However, when the product of the biosynthetic pathway begins to accumulate in the cell, removing the need for the cell to continue to make more, the expression of the operon is repressed. Conversely, **inducible operons**, like the *lac* operon of *E. coli*, often contain genes encoding enzymes in a pathway involved in the metabolism of a specific substrate like lactose. These enzymes are only required when that substrate is available, thus expression of the operons is typically induced only in the presence of the substrate.

The *trp* Operon: A Repressible Operon

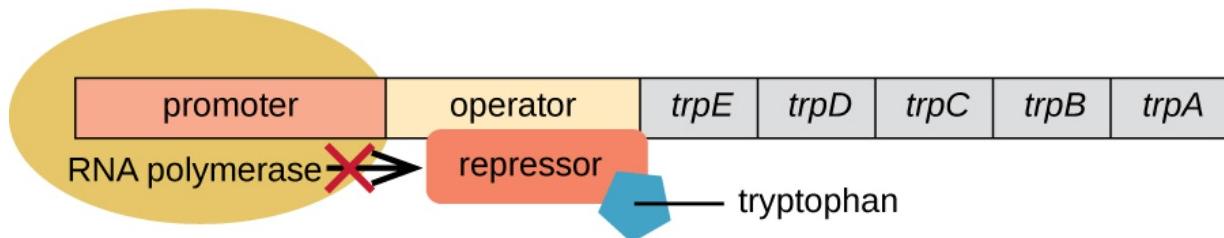
E. coli can synthesize tryptophan using enzymes that are encoded by five structural genes located next to each other in the *trp* operon ([\[link\]](#)). When environmental tryptophan is low, the operon is turned on. This means that transcription is initiated, the genes are expressed, and tryptophan is synthesized. However, if tryptophan is present in the environment, the *trp* operon is turned off. Transcription does not occur and tryptophan is not synthesized.

When tryptophan is not present in the cell, the repressor by itself does not bind to the operator; therefore, the operon is active and tryptophan is synthesized. However, when tryptophan accumulates in the cell, two tryptophan molecules bind to the *trp* repressor molecule, which changes its shape, allowing it to bind to the *trp* operator. This binding of the active form of the *trp* repressor to the operator blocks RNA polymerase from transcribing the structural genes, stopping expression of the operon. Thus, the actual product of the biosynthetic pathway controlled by the operon regulates the expression of the operon.

In the absence of tryptophan, the *trp* repressor dissociates from the operator, and RNA synthesis proceeds.



When tryptophan is present, the *trp* repressor binds the operator, and RNA synthesis is blocked.



The five structural genes needed to synthesize tryptophan in *E. coli* are located next to each other in the *trp* operon. When tryptophan is absent, the repressor protein does not bind to the operator, and the genes are transcribed. When tryptophan is plentiful, tryptophan binds the repressor protein at the operator sequence. This physically blocks the RNA polymerase from transcribing the tryptophan biosynthesis genes.

Note:



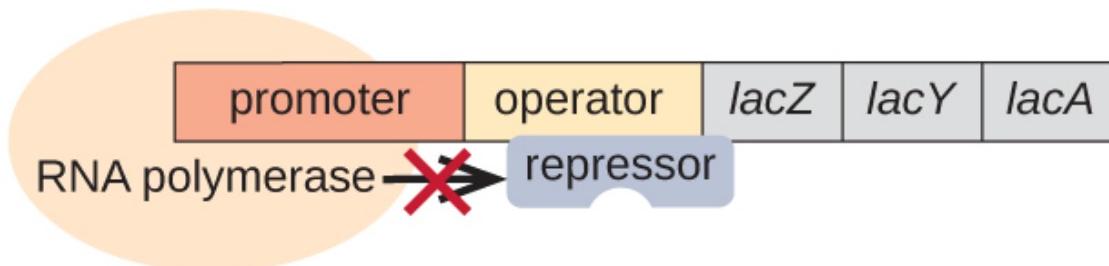
Watch this [video](#) to learn more about the *trp* operon.

The *lac* Operon: An Inducible Operon

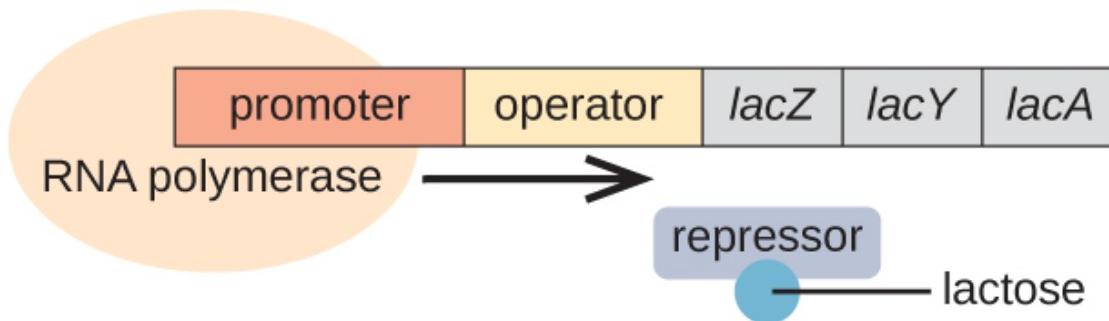
The *lac* operon is an example of an inducible operon that is also subject to activation in the absence of glucose ([\[link\]](#)). The *lac* operon encodes three structural genes necessary to acquire and process the disaccharide lactose from the environment, breaking it down into the simple sugars glucose and galactose. For the *lac* operon to be expressed, lactose must be present. This makes sense for the cell because it would be energetically wasteful to create the enzymes to process lactose if lactose was not available.

In the absence of lactose, the *lac* repressor is bound to the operator region of the *lac* operon, physically preventing RNA polymerase from transcribing the structural genes. However, when lactose is present, the lactose inside the cell is converted to allolactose. Allolactose serves as an inducer molecule, binding to the repressor and changing its shape so that it is no longer able to bind to the operator DNA. Removal of the repressor in the presence of lactose allows RNA polymerase to move through the operator region and begin transcription of the *lac* structural genes.

In the absence of lactose, the *lac* repressor binds the operator, and transcription is blocked.



In the presence of lactose, the *lac* repressor is released from the operator, and transcription proceeds at a slow rate.



The three structural genes that are needed to degrade lactose in *E. coli* are located next to each other in the *lac* operon. When lactose is absent, the repressor protein binds to the operator, physically blocking the RNA polymerase from transcribing the *lac* structural genes. When lactose is available, a lactose molecule binds the repressor protein, preventing the repressor from binding to the operator sequence, and the genes are transcribed.

The *lac* Operon: Activation by Catabolite Activator Protein

Bacteria typically have the ability to use a variety of substrates as carbon sources. However, because glucose is usually preferable to other substrates,

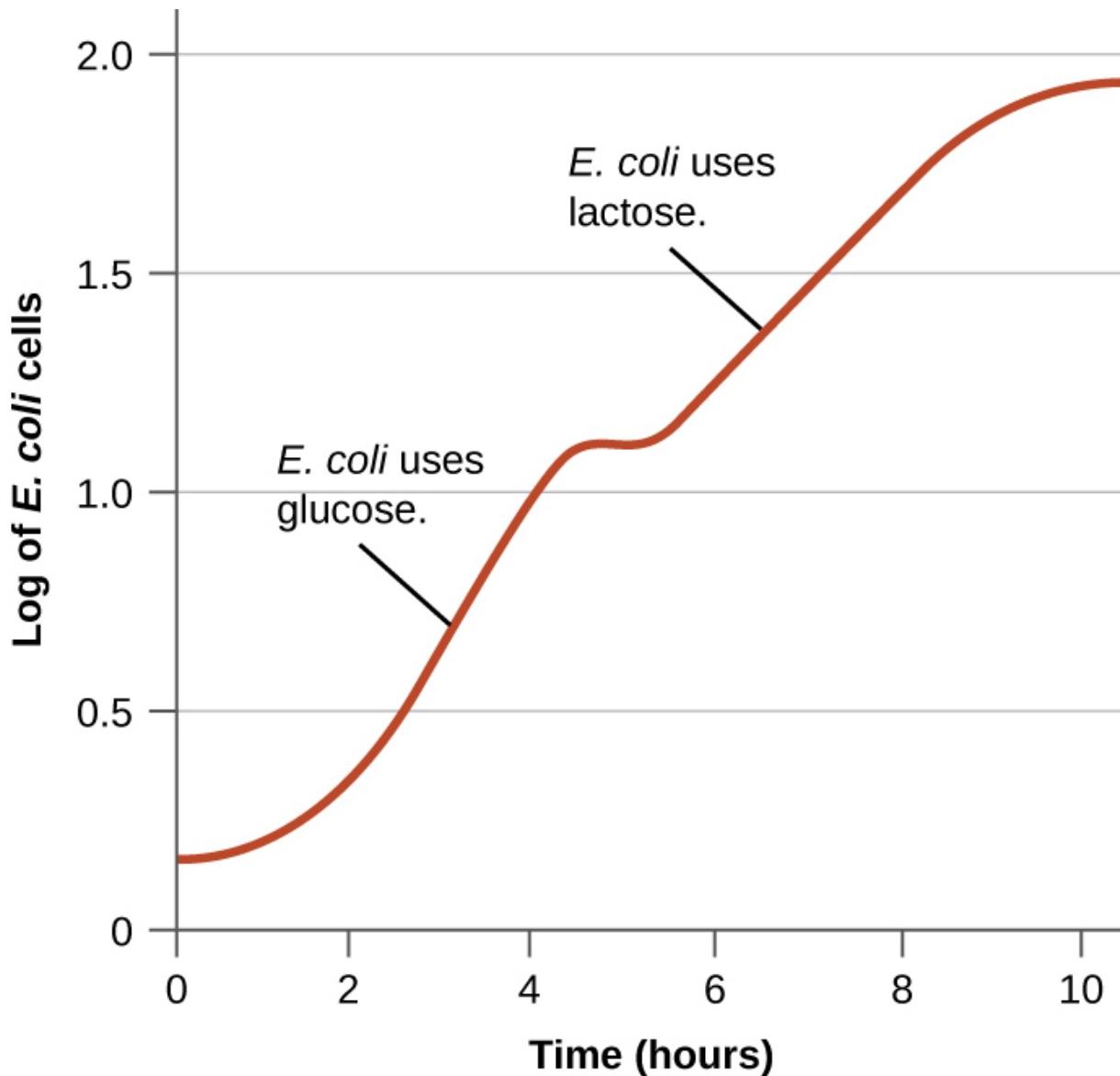
bacteria have mechanisms to ensure that alternative substrates are only used when glucose has been depleted. Additionally, bacteria have mechanisms to ensure that the genes encoding enzymes for using alternative substrates are expressed only when the alternative substrate is available. In the 1940s, Jacques Monod was the first to demonstrate the preference for certain substrates over others through his studies of *E. coli*'s growth when cultured in the presence of two different substrates simultaneously. Such studies generated diauxic growth curves, like the one shown in [\[link\]](#). Although the preferred substrate glucose is used first, *E. coli* grows quickly and the enzymes for lactose metabolism are absent. However, once glucose levels are depleted, growth rates slow, inducing the expression of the enzymes needed for the metabolism of the second substrate, lactose. Notice how the growth rate in lactose is slower, as indicated by the lower steepness of the growth curve.

The ability to switch from glucose use to another substrate like lactose is a consequence of the activity of an enzyme called Enzyme IIA (EIIA). When glucose levels drop, cells produce less ATP from catabolism (see [Catabolism of Carbohydrates](#)), and EIIA becomes phosphorylated.

Phosphorylated EIIA activates adenylyl cyclase, an enzyme that converts some of the remaining ATP to **cyclic AMP (cAMP)**, a cyclic derivative of AMP and important signaling molecule involved in glucose and energy metabolism in *E. coli*. As a result, cAMP levels begin to rise in the cell ([\[link\]](#)).

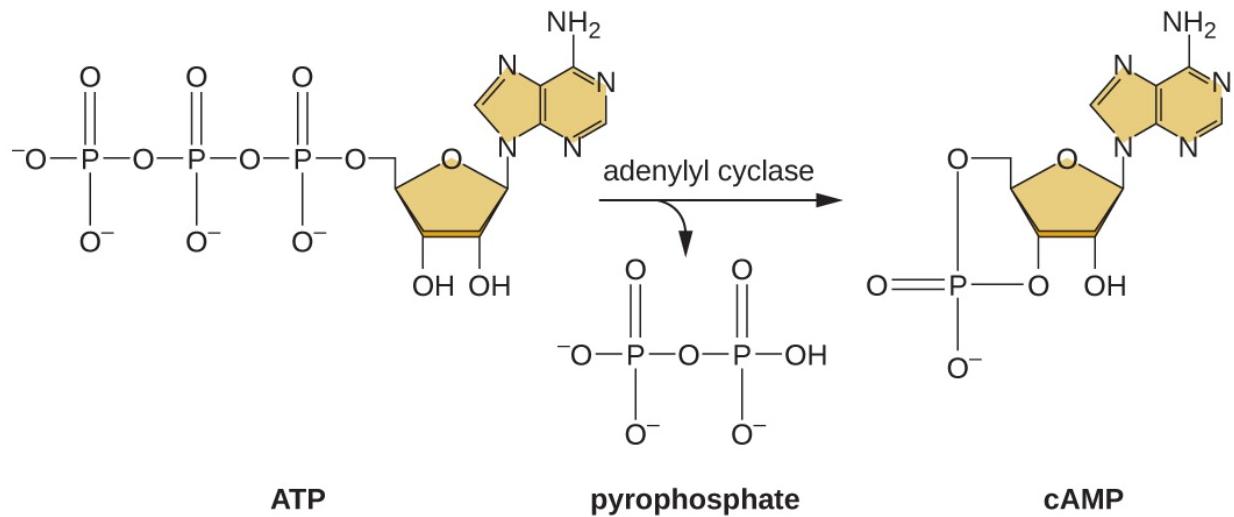
The *lac* operon also plays a role in this switch from using glucose to using lactose. When glucose is scarce, the accumulating cAMP caused by increased adenylyl cyclase activity binds to **catabolite activator protein (CAP)**, also known as cAMP receptor protein (CRP). The complex binds to the promoter region of the *lac* operon ([\[link\]](#)). In the regulatory regions of these operons, a CAP binding site is located upstream of the RNA polymerase binding site in the promoter. Binding of the CAP-cAMP complex to this site increases the binding ability of RNA polymerase to the promoter region to initiate the transcription of the structural genes. Thus, in the case of the *lac* operon, for transcription to occur, lactose must be present (removing the lac repressor protein) and glucose levels must be depleted (allowing binding of an activating protein). When glucose levels are high,

there is catabolite repression of operons encoding enzymes for the metabolism of alternative substrates. Because of low cAMP levels under these conditions, there is an insufficient amount of the CAP-cAMP complex to activate transcription of these operons. See [\[link\]](#) for a summary of the regulation of the lac operon.



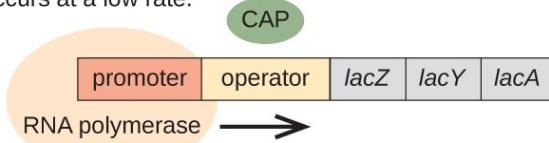
When grown in the presence of two substrates, *E. coli* uses the preferred substrate (in this case glucose) until it is depleted. Then,

enzymes needed for the metabolism of the second substrate are expressed and growth resumes, although at a slower rate.

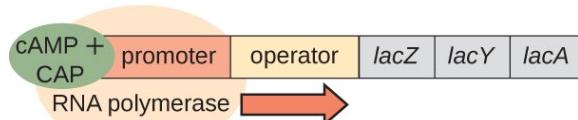


When ATP levels decrease due to depletion of glucose, some remaining ATP is converted to cAMP by adenylyl cyclase. Thus, increased cAMP levels signal glucose depletion.

In the absence of cAMP, CAP does not bind the promoter. Transcription occurs at a low rate.

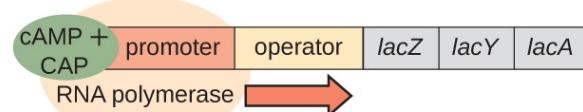


In the presence of cAMP, CAP binds the promoter and increases RNA polymerase activity.

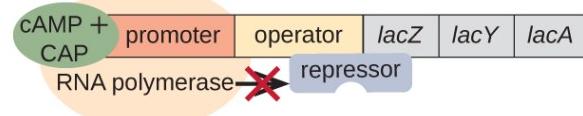


(a)

cAMP-CAP complex stimulates RNA polymerase activity and increases RNA synthesis.



However, even in the presence of cAMP-CAP complex, RNA synthesis is blocked when repressor is bound to the operator.



(b)

- (a) In the presence of cAMP, CAP binds to the promoters of operons, like the *lac* operon, that encode genes for enzymes for the use of alternate substrates. (b) For the *lac* operon to be expressed, there must be activation by cAMP-CAP as well as removal of the lac repressor from the operator.

Conditions Affecting Transcription of the *lac* Operon

Glucose	CAP binds	Lactose	Repressor binds	Transcription
+	-	-	+	No
+	-	+	-	Some

Conditions Affecting Transcription of the *lac* Operon

Glucose	CAP binds	Lactose	Repressor binds	Transcription
—	+	—	+	No
—	+	+	—	Yes

Note:



Watch an [animated tutorial](#) about the workings of lac operon here.

Note:

- What affects the binding of the *trp* operon repressor to the operator?
- How and when is the behavior of the *lac* repressor protein altered?
- In addition to being repressible, how else is the *lac* operon regulated?

Global Responses of Prokaryotes

In prokaryotes, there are also several higher levels of gene regulation that have the ability to control the transcription of many related operons simultaneously in response to an environmental signal. A group of operons all controlled simultaneously is called a regulon.

Alarmones

When sensing impending stress, prokaryotes alter the expression of a wide variety of operons to respond in coordination. They do this through the production of **alarmones**, which are small intracellular nucleotide derivatives. Alarmones change which genes are expressed and stimulate the expression of specific stress-response genes. The use of alarmones to alter gene expression in response to stress appears to be important in pathogenic bacteria. On encountering host defense mechanisms and other harsh conditions during infection, many operons encoding virulence genes are upregulated in response to alarmone signaling. Knowledge of these responses is key to being able to fully understand the infection process of many pathogens and to the development of therapies to counter this process.

Alternate σ Factors

Since the σ subunit of bacterial RNA polymerase confers specificity as to which promoters should be transcribed, altering the **σ factor** used is another way for bacteria to quickly and globally change what regulons are transcribed at a given time. The σ factor recognizes sequences within a bacterial promoter, so different σ factors will each recognize slightly different promoter sequences. In this way, when the cell senses specific environmental conditions, it may respond by changing which σ factor it expresses, degrading the old one and producing a new one to transcribe the operons encoding genes whose products will be useful under the new environmental condition. For example, in sporulating bacteria of the genera *Bacillus* and *Clostridium* (which include many pathogens), a group of σ

factors controls the expression of the many genes needed for sporulation in response to sporulation-stimulating signals.

Note:

- What is the name given to a collection of operons that can be regulated as a group?
- What type of stimulus would trigger the transcription of a different σ factor?

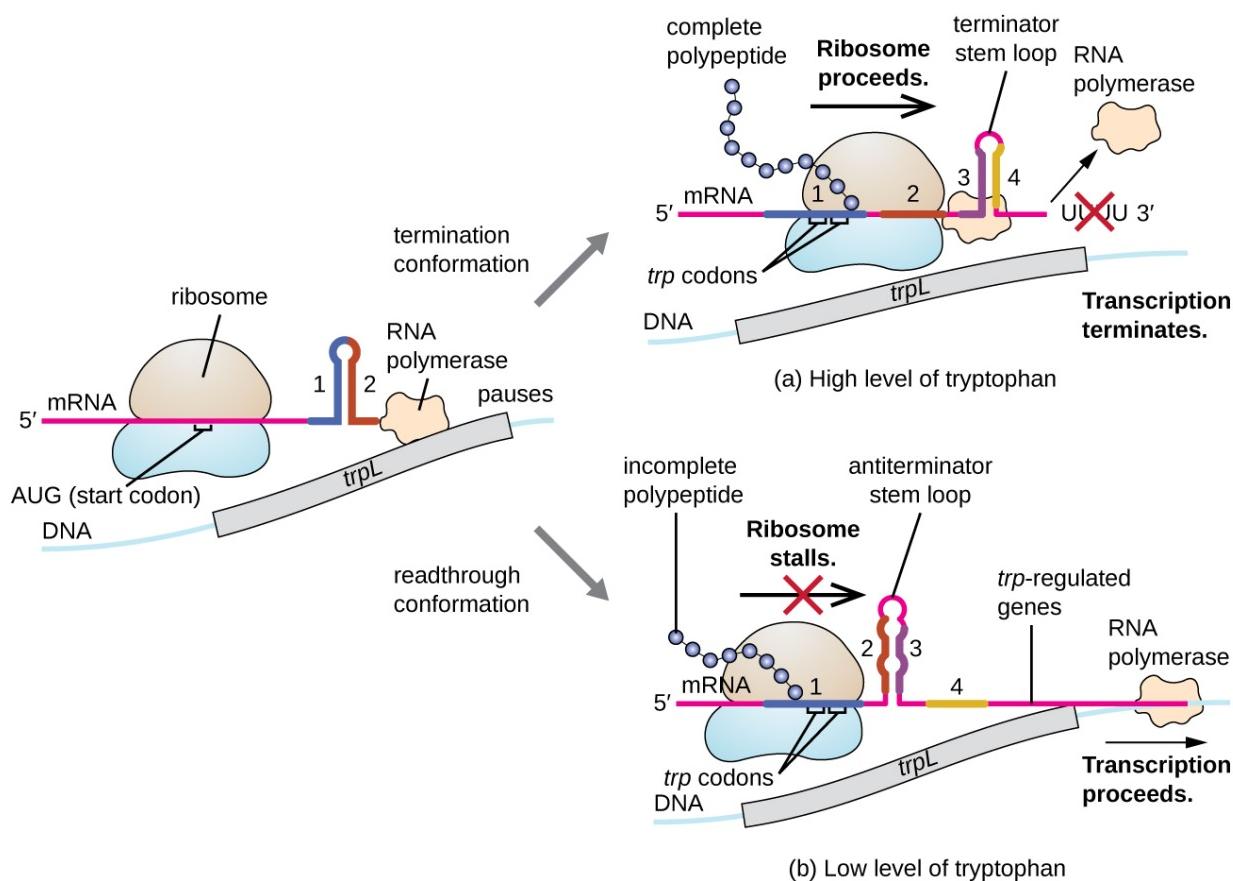
Additional Methods of Regulation in Bacteria: Attenuation and Riboswitches

Although most gene expression is regulated at the level of transcription initiation in prokaryotes, there are also mechanisms to control both the completion of transcription as well as translation concurrently. Since their discovery, these mechanisms have been shown to control the completion of transcription and translation of many prokaryotic operons. Because these mechanisms link the regulation of transcription and translation directly, they are specific to prokaryotes, because these processes are physically separated in eukaryotes.

One such regulatory system is **attenuation**, whereby secondary stem-loop structures formed within the 5' end of an mRNA being transcribed determine if transcription to complete the synthesis of this mRNA will occur and if this mRNA will be used for translation. Beyond the transcriptional repression mechanism already discussed, attenuation also controls expression of the *trp* operon in *E. coli* ([\[link\]](#)). The *trp* operon regulatory region contains a leader sequence called *trpL* between the operator and the first structural gene, which has four stretches of RNA that can base pair with each other in different combinations. When a terminator stem-loop forms, transcription terminates, releasing RNA polymerase from the mRNA. However, when an antiterminator stem-loop forms, this

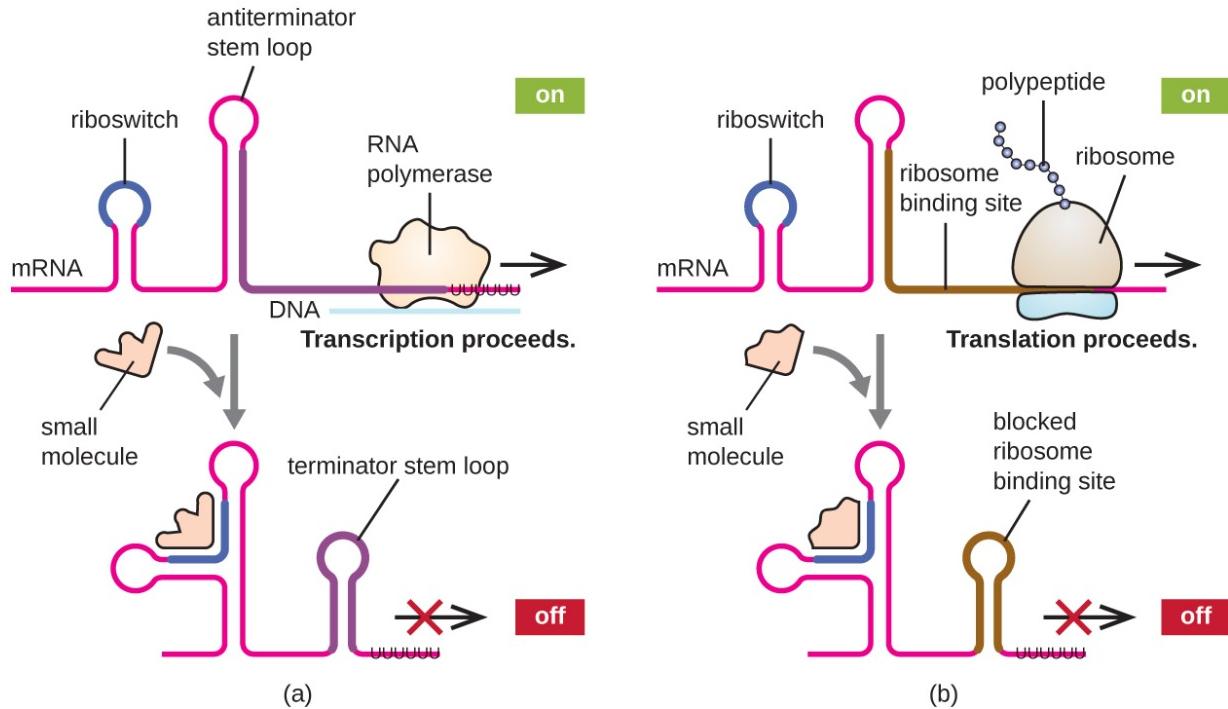
prevents the formation of the terminator stem-loop, so RNA polymerase can transcribe the structural genes.

A related mechanism of concurrent regulation of transcription and translation in prokaryotes is the use of a **riboswitch**, a small region of noncoding RNA found within the 5' end of some prokaryotic mRNA molecules ([\[link\]](#)). A riboswitch may bind to a small intracellular molecule to stabilize certain secondary structures of the mRNA molecule. The binding of the small molecule determines which stem-loop structure forms, thus influencing the completion of mRNA synthesis and protein synthesis.



When tryptophan is plentiful, translation of the short leader peptide encoded by *trpL* proceeds, the terminator loop between regions 3 and 4 forms, and transcription terminates. When tryptophan levels are depleted, translation of the short leader peptide stalls at region 1,

allowing regions 2 and 3 to form an antiterminator loop, and RNA polymerase can transcribe the structural genes of the *trp* operon.

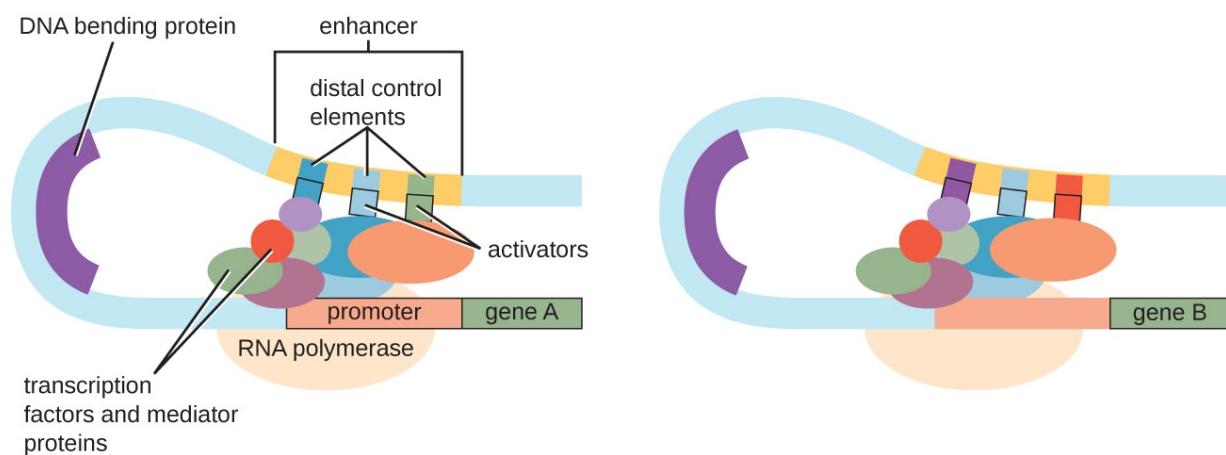


Riboswitches found within prokaryotic mRNA molecules can bind to small intracellular molecules, stabilizing certain RNA structures, influencing either the completion of the synthesis of the mRNA molecule itself (left) or the protein made using that mRNA (right).

Other Factors Affecting Gene Expression in Prokaryotes and Eukaryotes

Although the focus on our discussion of transcriptional control used prokaryotic operons as examples, eukaryotic transcriptional control is similar in many ways. As in prokaryotes, eukaryotic transcription can be controlled through the binding of transcription factors including repressors and activators. Interestingly, eukaryotic transcription can be influenced by

the binding of proteins to regions of DNA, called enhancers, rather far away from the gene, through DNA looping facilitated between the enhancer and the promoter ([\[link\]](#)). Overall, regulating transcription is a highly effective way to control gene expression in both prokaryotes and eukaryotes. However, the control of gene expression in eukaryotes in response to environmental and cellular stresses can be accomplished in additional ways without the binding of transcription factors to regulatory regions.



In eukaryotes, an enhancer is a DNA sequence that promotes transcription. Each enhancer is made up of short DNA sequences called distal control elements. Activators bound to the distal control elements interact with mediator proteins and transcription factors. Two different genes may have the same promoter but different distal control elements, enabling differential gene expression.

DNA-Level Control

In eukaryotes, the DNA molecules or associated histones can be chemically modified in such a way as to influence transcription; this is called **epigenetic regulation**. Methylation of certain cytosine nucleotides in DNA in response to environmental factors has been shown to influence use of

such DNA for transcription, with DNA methylation commonly correlating to lowered levels of gene expression. Additionally, in response to environmental factors, histone proteins for packaging DNA can also be chemically modified in multiple ways, including acetylation and deacetylation, influencing the packaging state of DNA and thus affecting the availability of loosely wound DNA for transcription. These chemical modifications can sometimes be maintained through multiple rounds of cell division, making at least some of these epigenetic changes heritable.

Note:



This [video](#) describes how epigenetic regulation controls gene expression.

Note:

- What stops or allows transcription to proceed when attenuation is operating?
- What determines the state of a riboswitch?
- Describe the function of an enhancer.
- Describe two mechanisms of epigenetic regulation in eukaryotes.

Key Concepts and Summary

- **Gene expression** is a tightly regulated process.
- Gene expression in prokaryotes is largely regulated at the point of transcription. Gene expression in eukaryotes is additionally regulated post-transcriptionally.
- Prokaryotic structural genes of related function are often organized into **operons**, all controlled by transcription from a single promoter. The regulatory region of an operon includes the promoter itself and the region surrounding the promoter to which transcription factors can bind to influence transcription.
- Although some operons are **constitutively expressed**, most are subject to regulation through the use of **transcription factors** (repressors and activators). A **repressor** binds to an **operator**, a DNA sequence within the regulatory region between the RNA polymerase binding site in the promoter and first structural gene, thereby physically blocking transcription of these operons. An **activator** binds within the regulatory region of an operon, helping RNA polymerase bind to the promoter, thereby enhancing the transcription of this operon. An **inducer** influences transcription through interacting with a repressor or activator.
- The *trp* operon is a classic example of a **repressible operon**. When tryptophan accumulates, tryptophan binds to a repressor, which then binds to the operator, preventing further transcription.
- The *lac* operon is a classic example an **inducible operon**. When lactose is present in the cell, it is converted to allolactose. Allolactose acts as an inducer, binding to the repressor and preventing the repressor from binding to the operator. This allows transcription of the structural genes.
- The *lac* operon is also subject to activation. When glucose levels are depleted, some cellular ATP is converted into cAMP, which binds to the **catabolite activator protein (CAP)**. The cAMP-CAP complex activates transcription of the *lac* operon. When glucose levels are high, its presence prevents transcription of the *lac* operon and other operons by **catabolite repression**.
- Small intracellular molecules called **alarmones** are made in response to various environmental stresses, allowing bacteria to control the transcription of a group of operons, called a regulon.

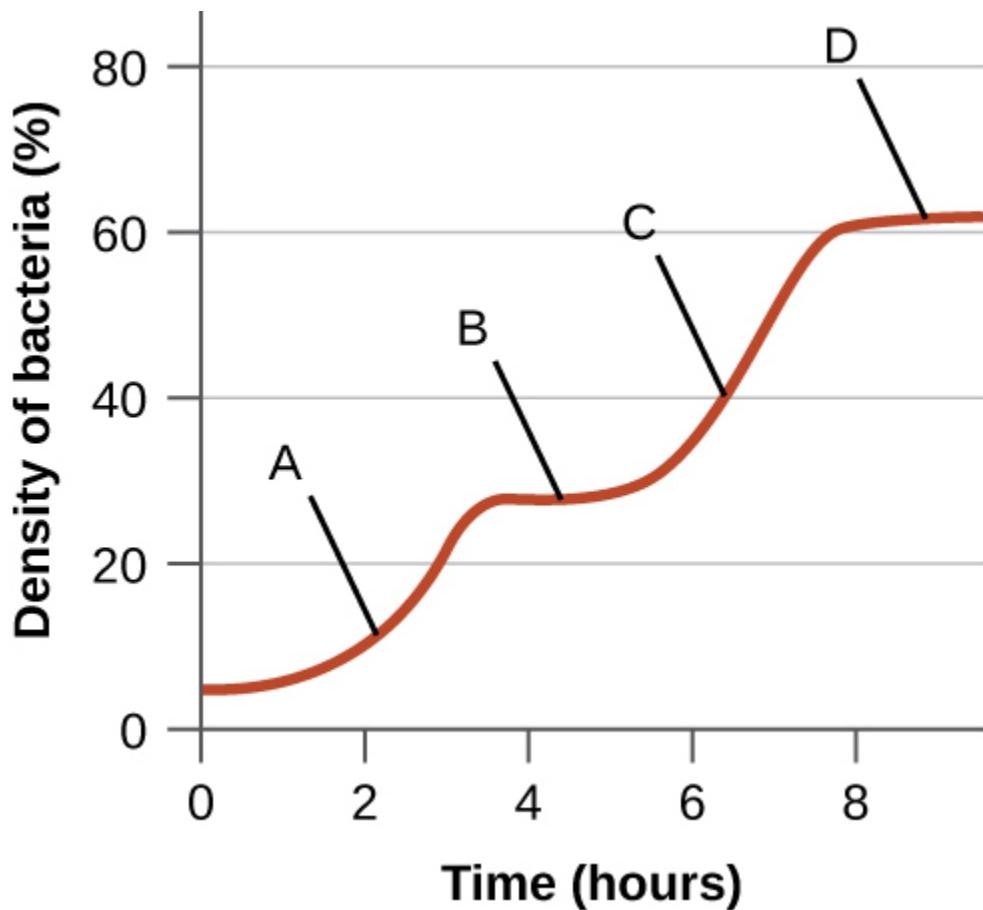
- Bacteria have the ability to change which **σ factor** of RNA polymerase they use in response to environmental conditions to quickly and globally change which regulons are transcribed.
- Prokaryotes have regulatory mechanisms, including **attenuation** and the use of **riboswitches**, to simultaneously control the completion of transcription and translation from that transcript. These mechanisms work through the formation of stem loops in the 5' end of an mRNA molecule currently being synthesized.
- There are additional points of regulation of gene expression in prokaryotes and eukaryotes. In eukaryotes, **epigenetic regulation** by chemical modification of DNA or histones, and regulation of RNA processing are two methods.

Critical Thinking

Exercise:

Problem:

The following figure is from Monod's original work on diauxic growth showing the growth of *E. coli* in the simultaneous presence of xylose and glucose as the only carbon sources. Explain what is happening at points A–D with respect to the carbon source being used for growth, and explain whether the xylose-use operon is being expressed (and why). Note that expression of the enzymes required for xylose use is regulated in a manner similar to the expression of the enzymes required for lactose use.



Introduction

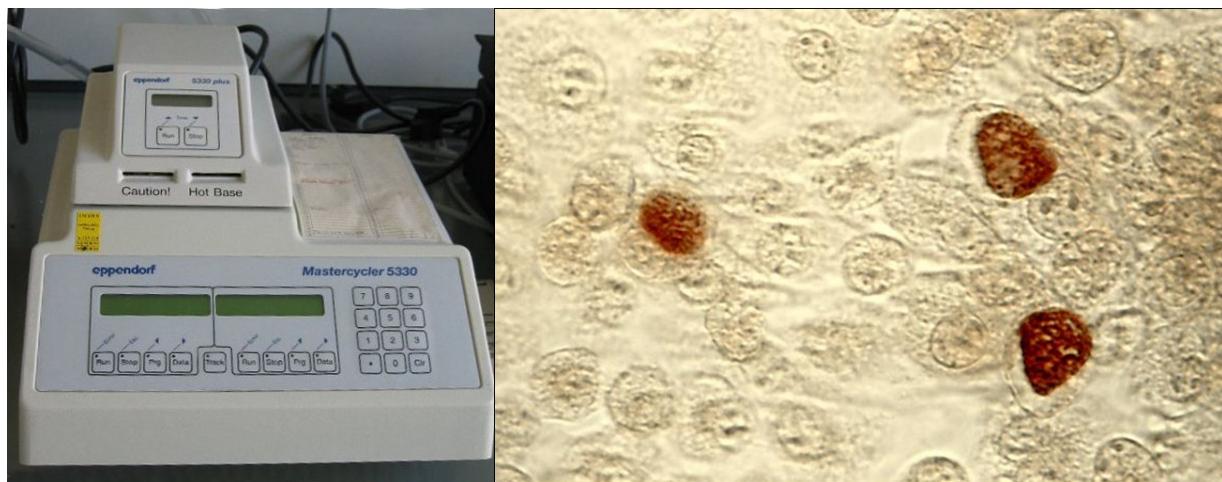
class="introduction"

A thermal cycler (left) is used during a polymerase chain reaction

(PCR). PCR amplifies the number of copies of DNA and can assist in diagnosis of infections caused by microbes that are difficult to culture, such as

Chlamydia trachomatis (right). *C. trachomatis* causes chlamydia, the most common sexually transmitted disease in the United States, and

trachoma,
the world's
leading
cause of
preventable
blindness.
(credit right:
modification
of work
by Centers
for Disease
Control and
Prevention)



Watson and Crick's identification of the structure of DNA in 1953 was the seminal event in the field of genetic engineering. Since the 1970s, there has been a veritable explosion in scientists' ability to manipulate DNA in ways that have revolutionized the fields of biology, medicine, diagnostics, forensics, and industrial manufacturing. Many of the molecular tools discovered in recent decades have been produced using prokaryotic microbes. In this chapter, we will explore some of those tools, especially as they relate to applications in medicine and health care.

As an example, the thermal cycler in [\[link\]](#) is used to perform a diagnostic technique called the polymerase chain reaction (PCR), which relies on

DNA polymerase enzymes from thermophilic bacteria. Other molecular tools, such as restriction enzymes and plasmids obtained from microorganisms, allow scientists to insert genes from humans or other organisms into microorganisms. The microorganisms are then grown on an industrial scale to synthesize products such as insulin, vaccines, and biodegradable polymers. These are just a few of the numerous applications of microbial genetics that we will explore in this chapter.

Microbes and the Tools of Genetic Engineering

LEARNING OBJECTIVES

- Identify tools of molecular genetics that are derived from microorganisms
- Describe the methods used to create recombinant DNA molecules
- Describe methods used to introduce DNA into prokaryotic cells
- List the types of genomic libraries and describe their uses
- Describe the methods used to introduce DNA into eukaryotic cells

Note:

Part 1

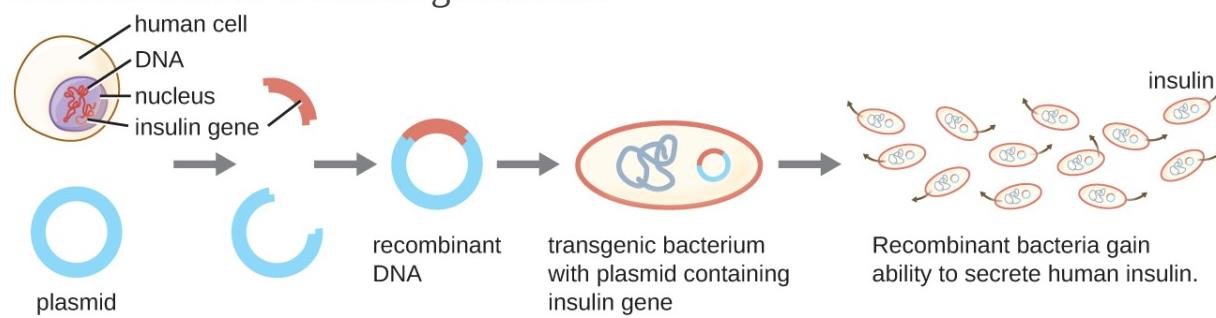
Kayla, a 24-year-old electrical engineer and running enthusiast, just moved from Arizona to New Hampshire to take a new job. On her weekends off, she loves to explore her new surroundings, going for long runs in the pine forests. In July she spent a week hiking through the mountains. In early August, Kayla developed a low fever, headache, and mild muscle aches, and she felt a bit fatigued. Not thinking much of it, she took some ibuprofen to combat her symptoms and vowed to get more rest.

- What types of medical conditions might be responsible for Kayla's symptoms?

Jump to the [next](#) Clinical Focus box.

The science of using living systems to benefit humankind is called **biotechnology**. Technically speaking, the domestication of plants and animals through farming and breeding practices is a type of biotechnology. However, in a contemporary sense, we associate biotechnology with the direct alteration of an organism's genetics to achieve desirable traits through the process of **genetic engineering**. Genetic engineering involves the use of **recombinant DNA technology**, the process by which a DNA sequence is manipulated *in vitro*, thus creating **recombinant DNA molecules** that have new combinations of genetic material. The recombinant DNA is then introduced into a host organism. If the DNA that is introduced comes from a different species, the host organism is now considered to be **transgenic**.

One example of a transgenic microorganism is the bacterial strain that produces human insulin ([\[link\]](#)). The insulin gene from humans was inserted into a plasmid. This recombinant DNA plasmid was then inserted into bacteria. As a result, these transgenic microbes are able to produce and secrete human insulin. Many prokaryotes are able to acquire foreign DNA and incorporate functional genes into their own genome through “mating” with other cells (conjugation), viral infection (transduction), and taking up DNA from the environment (transformation). Recall that these mechanisms are examples of horizontal gene transfer—the transfer of genetic material between cells of the same generation.



Recombinant DNA technology is the artificial recombination of DNA from two organisms. In this example, the human insulin gene is inserted into a bacterial plasmid. This recombinant plasmid can then be used to transform bacteria, which gain the ability to produce the insulin protein.

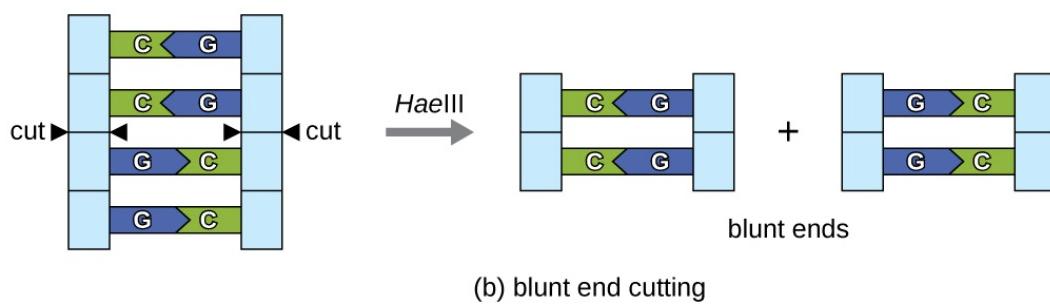
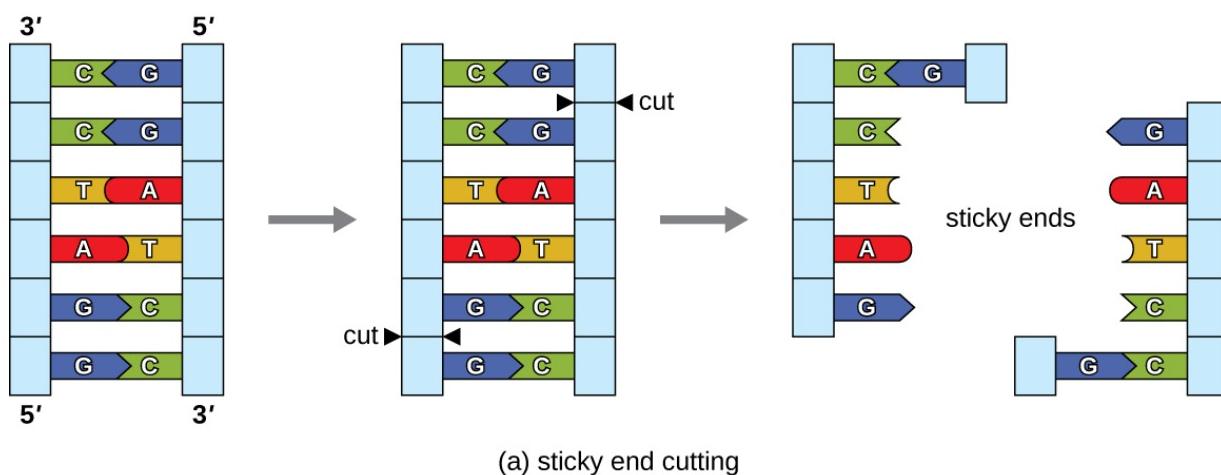
Molecular Cloning

Herbert Boyer and Stanley Cohen first demonstrated the complete **molecular cloning** process in 1973 when they successfully cloned genes from the African clawed frog (*Xenopus laevis*) into a bacterial plasmid that was then introduced into the bacterial host *Escherichia coli*. Molecular cloning is a set of methods used to construct recombinant DNA and incorporate it into a host organism; it makes use of a number of molecular tools that are derived from microorganisms.

Restriction Enzymes and Ligases

In recombinant DNA technology, DNA molecules are manipulated using naturally occurring enzymes derived mainly from bacteria and viruses. The creation of recombinant DNA molecules is possible due to the use of naturally occurring **restriction endonucleases (restriction enzymes)**, bacterial enzymes produced as a protection mechanism to cut and destroy foreign cytoplasmic DNA that is most commonly a result of bacteriophage infection. Stewart Linn and Werner Arber discovered restriction enzymes in their 1960s studies of how *E. coli* limits bacteriophage replication on infection. Today, we use restriction enzymes extensively for cutting DNA fragments that can then be spliced into another DNA molecule to form recombinant molecules. Each restriction enzyme cuts DNA at a characteristic , a specific, usually palindromic, DNA sequence typically between four to six base pairs in length. A palindrome is a sequence of letters that reads the same forward as backward. (The word “level” is an example of a palindrome.) Palindromic DNA sequences contain the same base sequences in the 5' to 3' direction on one strand as in the 5' to 3' direction on the complementary strand. A restriction enzyme recognizes the DNA palindrome and cuts each backbone at identical positions in the palindrome. Some restriction enzymes cut to produce molecules that have complementary overhangs () while others cut without generating such overhangs, instead producing ([\[link\]](#)).

Molecules with complementary sticky ends can easily **anneal**, or form hydrogen bonds between complementary bases, at their sticky ends. The annealing step allows **hybridization** of the single-stranded overhangs. Hybridization refers to the joining together of two complementary single strands of DNA. Blunt ends can also attach together, but less efficiently than sticky ends due to the lack of complementary overhangs facilitating the process. In either case, **ligation** by DNA ligase can then rejoin the two sugar-phosphate backbones of the DNA through covalent bonding, making the molecule a continuous double strand. In 1972, Paul Berg, a Stanford biochemist, was the first to produce a recombinant DNA molecule using this technique, combining the SV40 monkey virus with *E. coli* bacteriophage lambda to create a hybrid.



- (a) In this six-nucleotide restriction enzyme site, recognized by the enzyme *BamHI*, notice that the sequence reads the same in the 5' to 3' direction on both strands. This is known as a palindrome. The cutting

of the DNA by the restriction enzyme at the sites (indicated by the black arrows) produces DNA fragments with sticky ends. Another piece of DNA cut with the same restriction enzyme could attach to one of these sticky ends, forming a recombinant DNA molecule. (b) This four-nucleotide recognition site also exhibits a palindromic sequence.

The cutting of the DNA by the restriction enzyme *Hae*III at the indicated sites produces DNA fragments with blunt ends. Any other piece of blunt DNA could attach to one of the blunt ends produced, forming a recombinant DNA molecule.

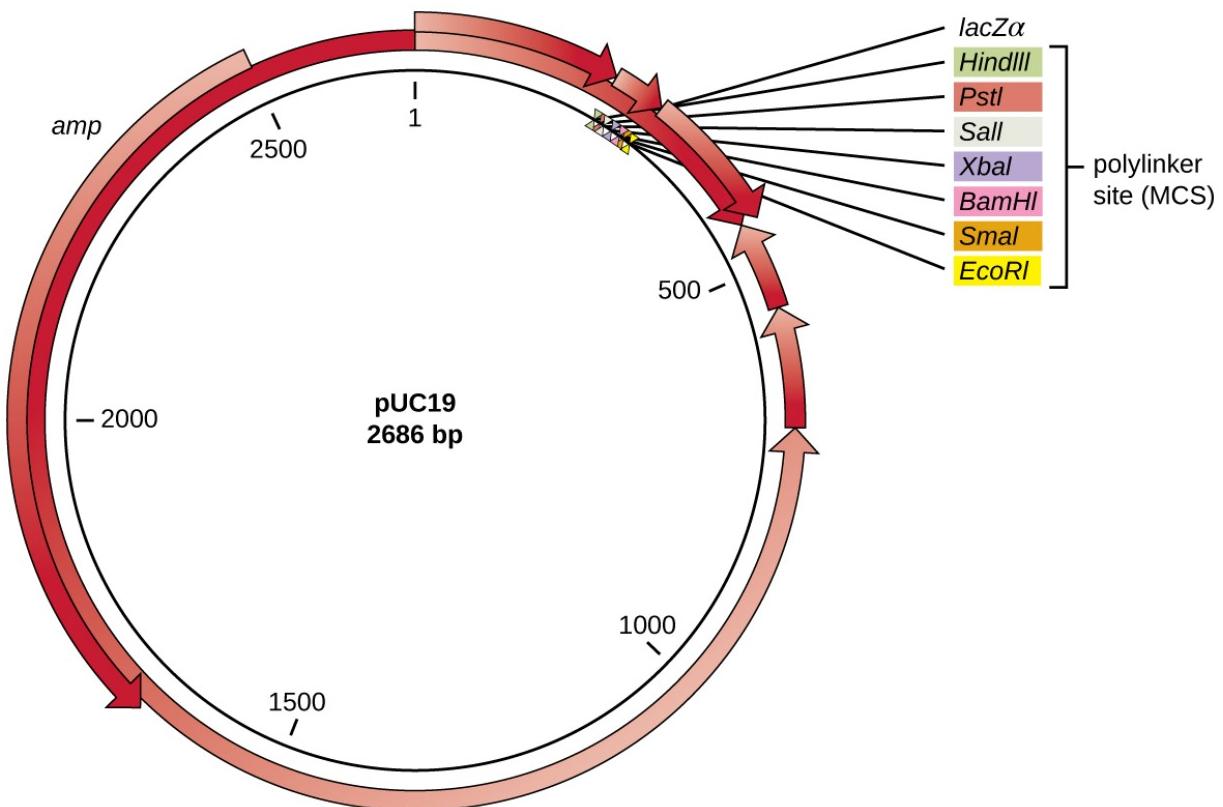
Plasmids

After restriction digestion, genes of interest are commonly inserted into plasmids, small pieces of typically circular, double-stranded DNA that replicate independently of the bacterial chromosome (see [Unique Characteristics of Prokaryotic Cells](#)). In recombinant DNA technology, plasmids are often used as **vectors**, DNA molecules that carry DNA fragments from one organism to another. Plasmids used as vectors can be genetically engineered by researchers and scientific supply companies to have specialized properties, as illustrated by the commonly used plasmid vector pUC19 ([\[link\]](#)). Some plasmid vectors contain genes that confer antibiotic resistance; these resistance genes allow researchers to easily find plasmid-containing colonies by plating them on media containing the corresponding antibiotic. The antibiotic kills all host cells that do not harbor the desired plasmid vector, but those that contain the vector are able to survive and grow.

Plasmid vectors used for cloning typically have a **polylinker site**, or **multiple cloning site (MCS)**. A polylinker site is a short sequence containing multiple unique restriction enzyme recognition sites that are used for inserting DNA into the plasmid after restriction digestion of both the DNA and the plasmid. Having these multiple restriction enzyme recognition sites within the polylinker site makes the plasmid vector

versatile, so it can be used for many different cloning experiments involving different restriction enzymes.

This polylinker site is often found within a **reporter gene**, another gene sequence artificially engineered into the plasmid that encodes a protein that allows for visualization of DNA insertion. The reporter gene allows a researcher to distinguish host cells that contain recombinant plasmids with cloned DNA fragments from host cells that only contain the non-recombinant plasmid vector. The most common reporter gene used in plasmid vectors is the bacterial *lacZ* gene encoding beta-galactosidase, an enzyme that naturally degrades lactose but can also degrade a colorless synthetic analog X-gal, thereby producing blue colonies on X-gal-containing media. The *lacZ* reporter gene is disabled when the recombinant DNA is spliced into the plasmid. Because the LacZ protein is not produced when the gene is disabled, X-gal is not degraded and white colonies are produced, which can then be isolated. This **blue-white screening** method is described later and shown in [\[link\]](#). In addition to these features, some plasmids come pre-digested and with an enzyme linked to the linearized plasmid to aid in ligation after the insertion of foreign DNA fragments.



The artificially constructed plasmid vector pUC19 is commonly used for cloning foreign DNA. Arrows indicate the directions in which the genes are transcribed. Note the polylinker site, containing multiple unique restriction enzyme recognition sites, found within the *lacZ* reporter gene. Also note the ampicillin (*amp*) resistance gene encoded on the plasmid.

Molecular Cloning using Transformation

The most commonly used mechanism for introducing engineered plasmids into a bacterial cell is transformation, a process in which bacteria take up free DNA from their surroundings. In nature, free DNA typically comes from other lysed bacterial cells; in the laboratory, free DNA in the form of recombinant plasmids is introduced to the cell's surroundings.

Some bacteria, such as *Bacillus* spp., are naturally competent, meaning they are able to take up foreign DNA. However, not all bacteria are naturally competent. In most cases, bacteria must be made artificially competent in the laboratory by increasing the permeability of the cell membrane. This can be achieved through chemical treatments that neutralize charges on the cell membrane or by exposing the bacteria to an electric field that creates microscopic pores in the cell membrane. These methods yield chemically competent or electrocompetent bacteria, respectively.

Following the transformation protocol, bacterial cells are plated onto an antibiotic-containing medium to inhibit the growth of the many host cells that were not transformed by the plasmid conferring antibiotic resistance. A technique called **blue-white screening** is then used for *lacZ*-encoding plasmid vectors such as pUC19. Blue colonies have a functional beta-galactosidase enzyme because the *lacZ* gene is uninterrupted, with no foreign DNA inserted into the polylinker site. These colonies typically result from the digested, linearized plasmid religating to itself. However, white colonies lack a functional beta-galactosidase enzyme, indicating the insertion of foreign DNA within the polylinker site of the plasmid vector, thus disrupting the *lacZ* gene. Thus, white colonies resulting from this blue-white screening contain plasmids with an insert and can be further screened to characterize the foreign DNA. To be sure the correct DNA was incorporated into the plasmid, the DNA insert can then be sequenced.

Note:



View an [animation of molecular cloning](#) from the DNA Learning Center.

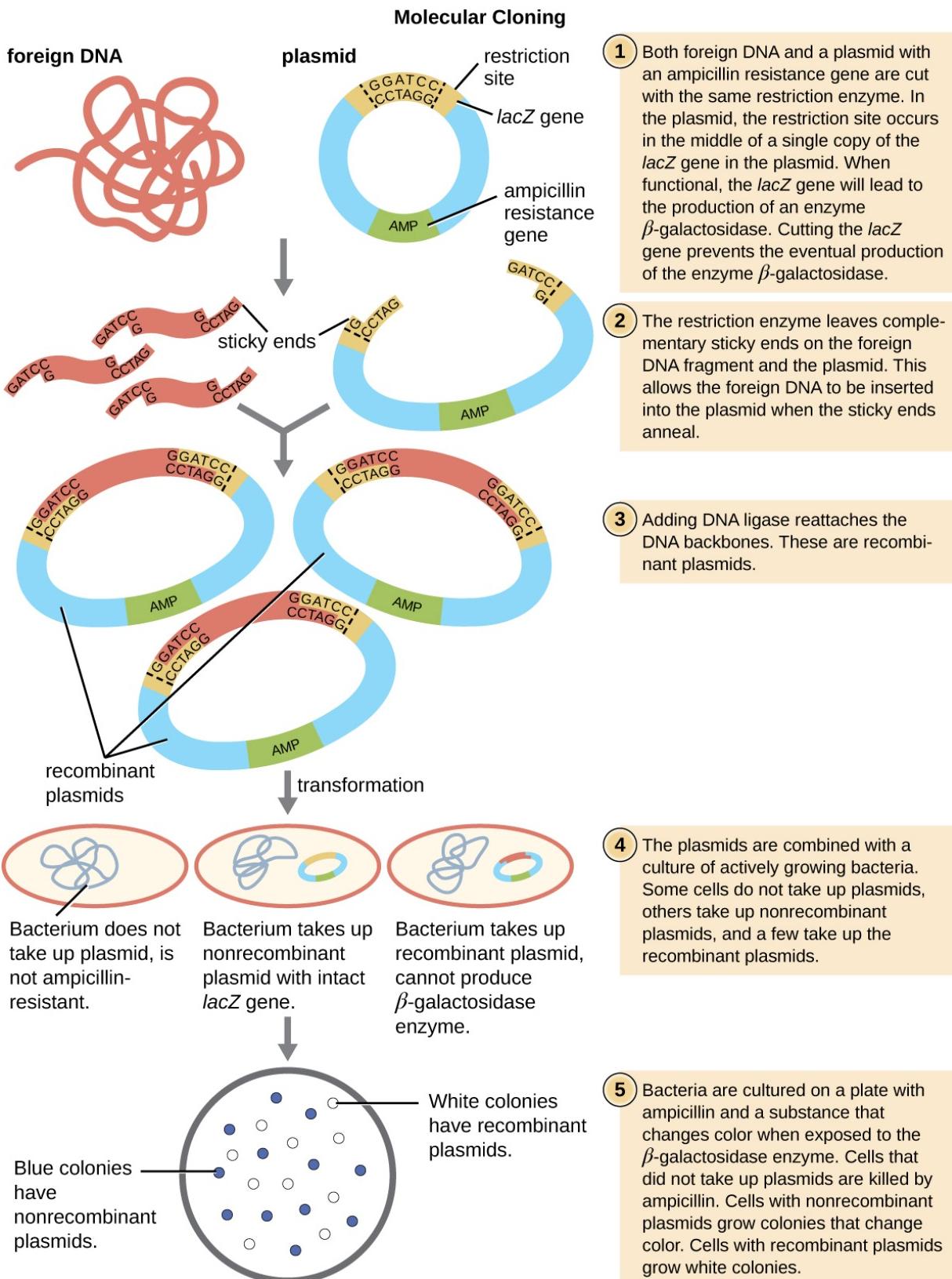
Note:

- In blue-white screening, what does a blue colony mean and why is it blue?

Molecular Cloning Using Conjugation or Transduction

The bacterial process of conjugation (see [How Asexual Prokaryotes Achieve Genetic Diversity](#)) can also be manipulated for molecular cloning. F plasmids, or fertility plasmids, are transferred between bacterial cells through the process of conjugation. Recombinant DNA can be transferred by conjugation when bacterial cells containing a recombinant F plasmid are mixed with compatible bacterial cells lacking the plasmid. F plasmids encode a surface structure called an F pilus that facilitates contact between a cell containing an F plasmid and one without an F plasmid. On contact, a cytoplasmic bridge forms between the two cells and the F-plasmid-containing cell replicates its plasmid, transferring a copy of the recombinant F plasmid to the recipient cell. Once it has received the recombinant F plasmid, the recipient cell can produce its own F pilus and facilitate transfer of the recombinant F plasmid to an additional cell. The use of conjugation to transfer recombinant F plasmids to recipient cells is another effective way to introduce recombinant DNA molecules into host cells.

Alternatively, bacteriophages can be used to introduce recombinant DNA into host bacterial cells through a manipulation of the transduction process (see [How Asexual Prokaryotes Achieve Genetic Diversity](#)). In the laboratory, DNA fragments of interest can be engineered into **phagemids**, which are plasmids that have phage sequences that allow them to be packaged into bacteriophages. Bacterial cells can then be infected with these bacteriophages so that the recombinant phagemids can be introduced into the bacterial cells. Depending on the type of phage, the recombinant DNA may be integrated into the host bacterial genome (lysogeny), or it may exist as a plasmid in the host's cytoplasm.



The steps involved in molecular cloning using bacterial transformation

are outlined in this graphic flowchart.

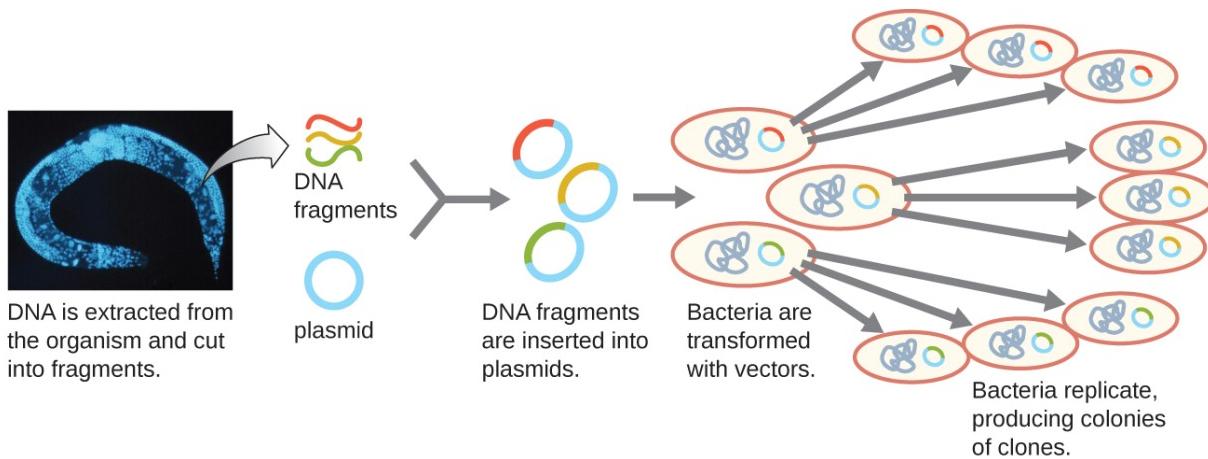
Note:

- What is the original function of a restriction enzyme?
- What two processes are exploited to get recombinant DNA into a bacterial host cell?
- Distinguish the uses of an antibiotic resistance gene and a reporter gene in a plasmid vector.

Creating a Genomic Library

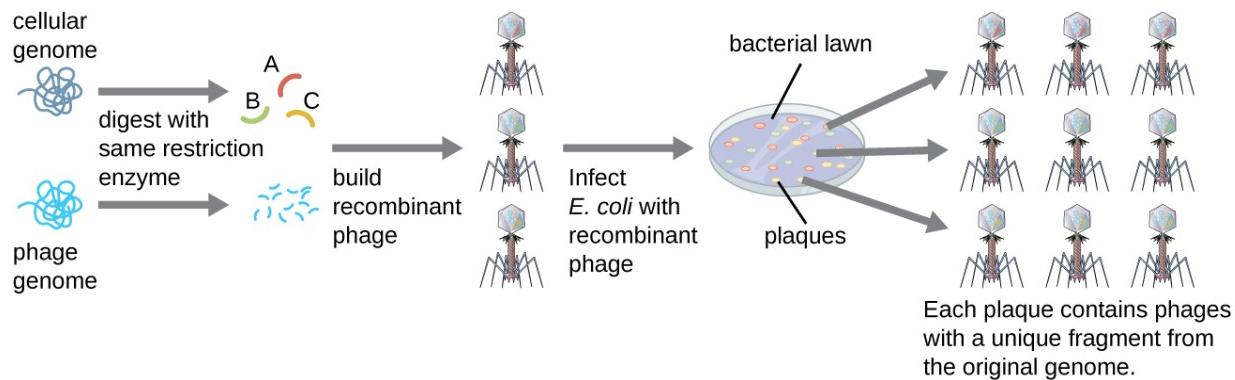
Molecular cloning may also be used to generate a **genomic library**. The library is a complete (or nearly complete) copy of an organism's genome contained as recombinant DNA plasmids engineered into unique clones of bacteria. Having such a library allows a researcher to create large quantities of each fragment by growing the bacterial host for that fragment. These fragments can be used to determine the sequence of the DNA and the function of any genes present.

One method for generating a genomic library is to ligate individual restriction enzyme-digested genomic fragments into plasmid vectors cut with the same restriction enzyme ([\[link\]](#)). After transformation into a bacterial host, each transformed bacterial cell takes up a single recombinant plasmid and grows into a colony of cells. All of the cells in this colony are identical **clones** and carry the same recombinant plasmid. The resulting library is a collection of colonies, each of which contains a fragment of the original organism's genome, that are each separate and distinct and can each be used for further study. This makes it possible for researchers to screen these different clones to discover the one containing a gene of interest from the original organism's genome.



The generation of a genomic library facilitates the discovery of the genomic DNA fragment that contains a gene of interest. (credit “micrograph”: modification of work by National Institutes of Health)

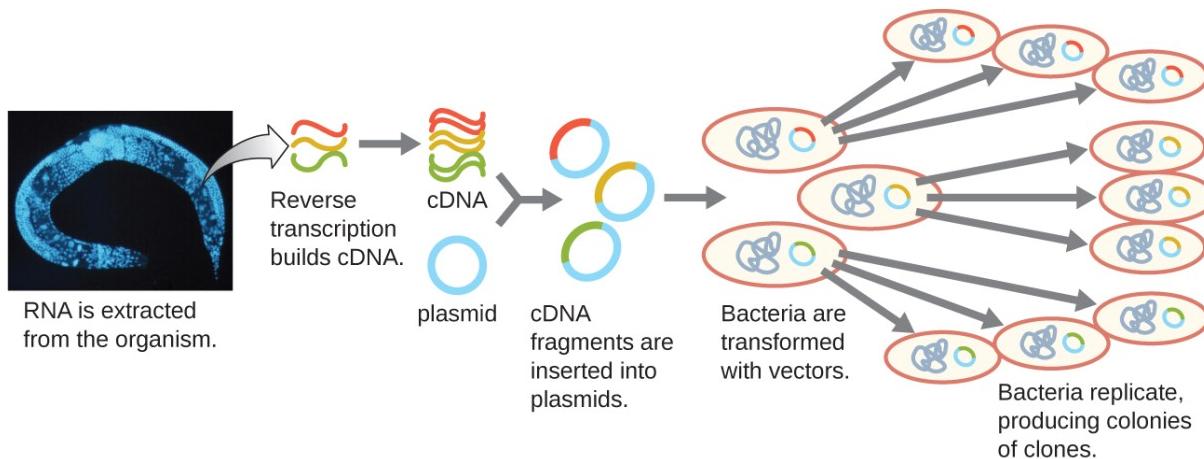
To construct a genomic library using larger fragments of genomic DNA, an *E. coli* bacteriophage, such as lambda, can be used as a host ([\[link\]](#)). Genomic DNA can be sheared or enzymatically digested and ligated into a pre-digested bacteriophage lambda DNA vector. Then, these recombinant phage DNA molecules can be packaged into phage particles and used to infect *E. coli* host cells on a plate. During infection within each cell, each recombinant phage will make many copies of itself and lyse the *E. coli* lawn, forming a plaque. Thus, each plaque from a phage library represents a unique recombinant phage containing a distinct genomic DNA fragment. Plaques can then be screened further to look for genes of interest. One advantage to producing a library using phages instead of plasmids is that a phage particle holds a much larger insert of foreign DNA compared with a plasmid vector, thus requiring a much smaller number of cultures to fully represent the entire genome of the original organism.



Recombinant phage DNA molecules are made by ligating digested phage particles with fragmented genomic DNA molecules. These recombinant phage DNA molecules are packaged into phage particles and allowed to infect a bacterial lawn. Each plaque represents a unique recombinant DNA molecule that can be further screened for genes of interest.

To focus on the expressed genes in an organism or even a tissue, researchers construct libraries using the organism's messenger RNA (mRNA) rather than its genomic DNA. Whereas all cells in a single organism will have the same genomic DNA, different tissues express different genes, producing different complements of mRNA. For example, all human cells' genomic DNA contains the gene for insulin, but only cells in the pancreas express mRNA directing the production of insulin. Because mRNA cannot be cloned directly, in the laboratory mRNA must be used as a template by the retroviral enzyme reverse transcriptase to make **complementary DNA (cDNA)**. A cell's full complement of mRNA can be reverse-transcribed into cDNA molecules, which can be used as a template for DNA polymerase to make double-stranded DNA copies; these fragments can subsequently be ligated into either plasmid vectors or bacteriophage to produce a cDNA library. The benefit of a cDNA library is that it contains DNA from only the expressed genes in the cell. This means that the introns, control sequences such as promoters, and DNA not destined to be translated into proteins are not represented in the library. The focus on translated sequences means that the library cannot be used to study the sequence and structure of the

genome in its entirety. The construction of a cDNA genomic library is shown in [\[link\]](#).



Complementary DNA (cDNA) is made from mRNA by the retroviral enzyme reverse transcriptase, converted into double-stranded copies, and inserted into either plasmid vectors or bacteriophage, producing a cDNA library. (credit “micrograph”: modification of work by National Institutes of Health)

Note:

- What are the hosts for the genomic libraries described?
- What is cDNA?

Introducing Recombinant Molecules into Eukaryotic Hosts

The use of bacterial hosts for genetic engineering laid the foundation for recombinant DNA technology; however, researchers have also had great

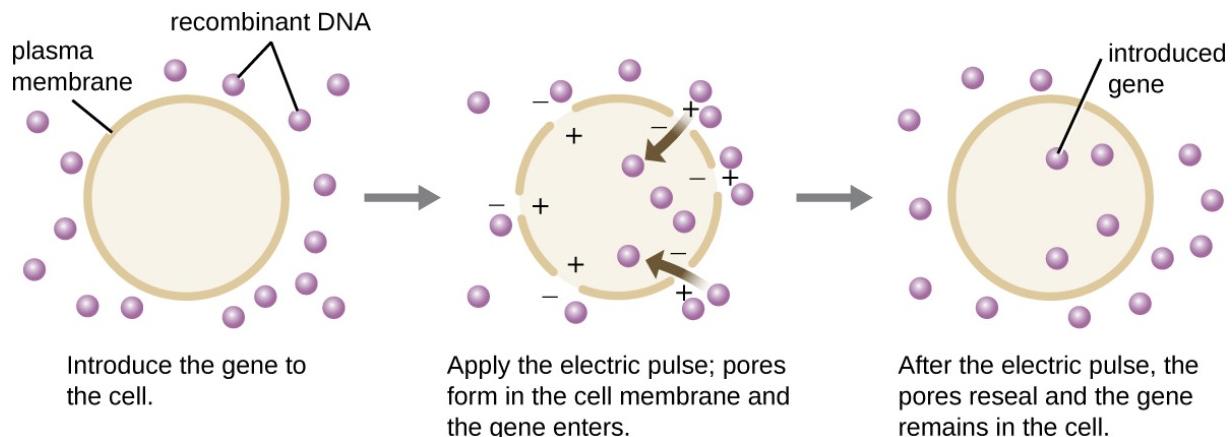
interest in genetically engineering eukaryotic cells, particularly those of plants and animals. The introduction of recombinant DNA molecules into eukaryotic hosts is called **transfection**. Genetically engineered plants, called transgenic plants, are of significant interest for agricultural and pharmaceutical purposes. The first transgenic plant sold commercially was the Flavr Savr delayed-ripening tomato, which came to market in 1994. Genetically engineered livestock have also been successfully produced, resulting, for example, in pigs with increased nutritional value [[footnote](#)] and goats that secrete pharmaceutical products in their milk. [[footnote](#)]

Liangxue Lai, Jing X. Kang, Rongfeng Li, Jingdong Wang, William T. Witt, Hwan Yul Yong, Yanhong Hao et al. “Generation of Cloned Transgenic Pigs Rich in Omega-3 Fatty Acids.” *Nature Biotechnology* 24 no. 4 (2006): 435–436.

Raylene Ramos Moura, Luciana Magalhães Melo, and Vicente José de Figueirêdo Freitas. “Production of Recombinant Proteins in Milk of Transgenic and Non-Transgenic Goats.” *Brazilian Archives of Biology and Technology* 54 no. 5 (2011): 927–938.

Electroporation

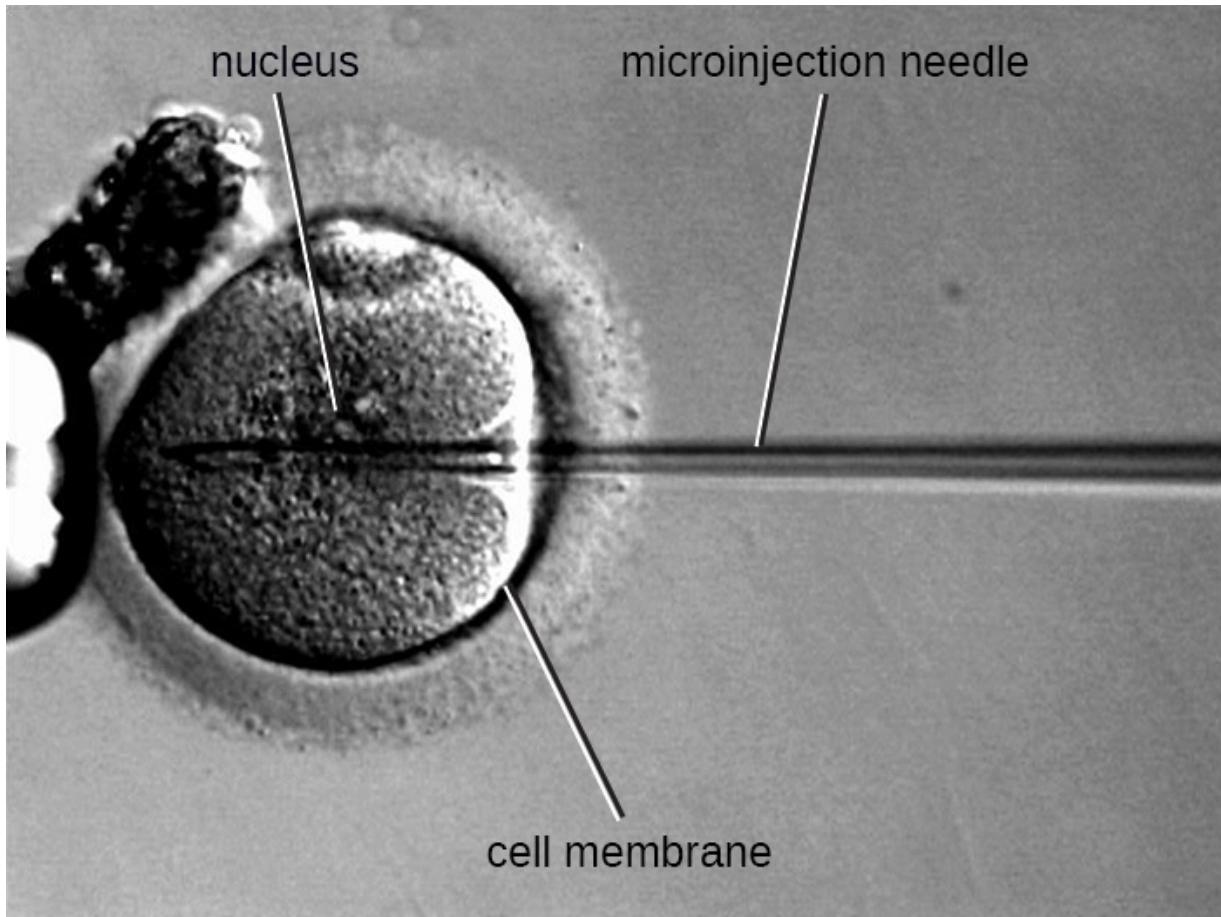
Compared to bacterial cells, eukaryotic cells tend to be less amenable as hosts for recombinant DNA molecules. Because eukaryotes are typically neither competent to take up foreign DNA nor able to maintain plasmids, transfection of eukaryotic hosts is far more challenging and requires more intrusive techniques for success. One method used for transfecting cells in cell culture is called **electroporation**. A brief electric pulse induces the formation of transient pores in the phospholipid bilayers of cells through which the gene can be introduced. At the same time, the electric pulse generates a short-lived positive charge on one side of the cell’s interior and a negative charge on the opposite side; the charge difference draws negatively charged DNA molecules into the cell ([\[link\]](#)).



Electroporation is one laboratory technique used to introduce DNA into eukaryotic cells.

Microinjection

An alternative method of transfection is called **microinjection**. Because eukaryotic cells are typically larger than those of prokaryotes, DNA fragments can sometimes be directly injected into the cytoplasm using a glass micropipette, as shown in [\[link\]](#).

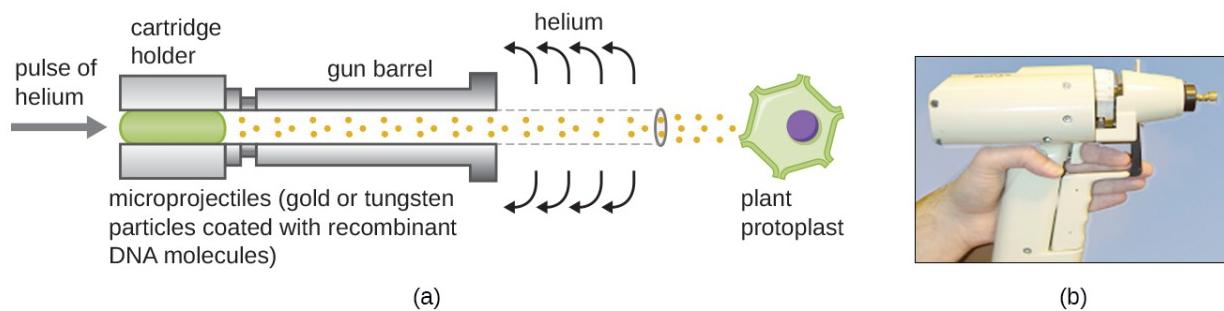


Microinjection is another technique for introducing DNA into eukaryotic cells. A microinjection needle containing recombinant DNA is able to penetrate both the cell membrane and nuclear envelope.

Gene Guns

Transfected plant cells can be even more difficult than animal cells because of their thick cell walls. One approach involves treating plant cells with enzymes to remove their cell walls, producing protoplasts. Then, a **gene gun** is used to shoot gold or tungsten particles coated with recombinant DNA molecules into the plant protoplasts at high speeds. Recipient

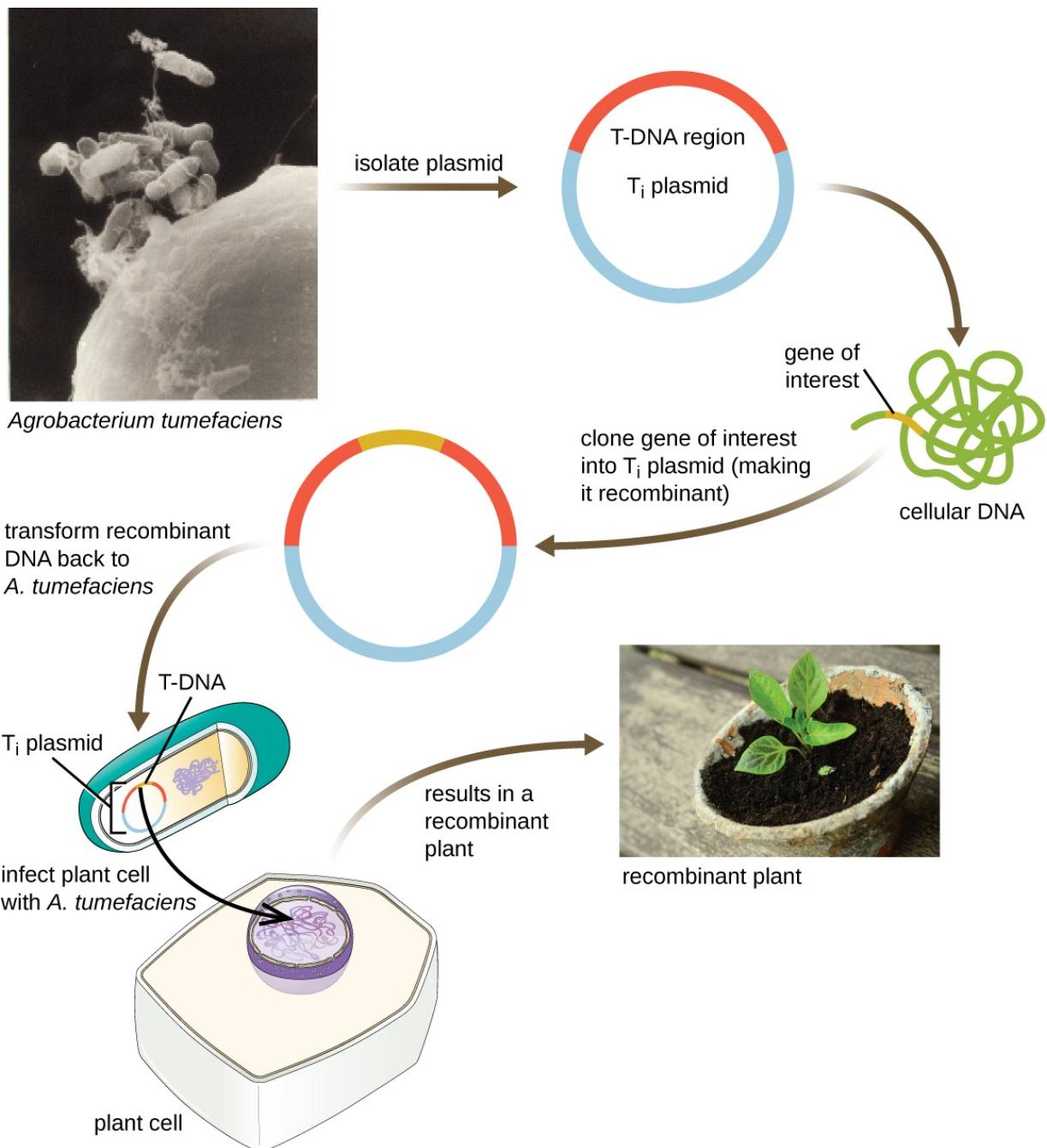
protoplast cells can then recover and be used to generate new transgenic plants ([\[link\]](#)).



Heavy-metal particles coated with recombinant DNA are shot into plant protoplasts using a gene gun. The resulting transformed cells are allowed to recover and can be used to generate recombinant plants. (a) A schematic of a gene gun. (b) A photograph of a gene gun. (credit a, b: modification of work by JA O'Brien, SC Lummis)

Shuttle Vectors

Another method of transfected plants involves **shuttle vectors**, plasmids that can move between bacterial and eukaryotic cells. The **tumor-inducing (Ti) plasmids** originating from the bacterium *Agrobacterium tumefaciens* are commonly used as shuttle vectors for incorporating genes into plants ([\[link\]](#)). In nature, the Ti plasmids of *A. tumefaciens* cause plants to develop tumors when they are transferred from bacterial cells to plant cells. Researchers have been able to manipulate these naturally occurring plasmids to remove their tumor-causing genes and insert desirable DNA fragments. The resulting recombinant Ti plasmids can be transferred into the plant genome through the natural transfer of Ti plasmids from the bacterium to the plant host. Once inside the plant host cell, the gene of interest recombines into the plant cell's genome.



The T_i plasmid of *Agrobacterium tumefaciens* is a useful shuttle vector for the uptake of genes of interest into plant cells. The gene of interest is cloned into the T_i plasmid, which is then introduced into plant cells.

The gene of interest then recombines into the plant cell's genome, allowing for the production of transgenic plants.

Viral Vectors

Viral vectors can also be used to transfect eukaryotic cells. In fact, this method is often used in gene therapy (see [Gene Therapy](#)) to introduce healthy genes into human patients suffering from diseases that result from genetic mutations. Viral genes can be deleted and replaced with the gene to be delivered to the patient; [footnote] the virus then infects the host cell and delivers the foreign DNA into the genome of the targeted cell.

Adenoviruses are often used for this purpose because they can be grown to high titer and can infect both nondividing and dividing host cells. However, use of viral vectors for gene therapy can pose some risks for patients, as discussed in [Gene Therapy](#).

William S.M. Wold and Karoly Toth. “Adenovirus Vectors for Gene Therapy, Vaccination and Cancer Gene Therapy.” *Current Gene Therapy* 13 no. 6 (2013): 421.

Note:

- What are the methods used to introduce recombinant DNA vectors into animal cells?
- Compare and contrast shuttle vectors and viral vectors.

Key Concepts and Summary

- **Biotechnology** is the science of utilizing living systems to benefit humankind. In recent years, the ability to directly alter an organism's genome through **genetic engineering** has been made possible due to advances in **recombinant DNA technology**, which allows researchers to create **recombinant DNA molecules** with new combinations of genetic material.
- **Molecular cloning** involves methods used to construct recombinant DNA and facilitate their replication in host organisms. These methods include the use of **restriction enzymes** (to cut both foreign DNA and

plasmid vectors), ligation (to paste fragments of DNA together), and the introduction of recombinant DNA into a host organism (often bacteria).

- **Blue-white screening** allows selection of bacterial transformants that contain recombinant plasmids using the phenotype of a **reporter gene** that is disabled by insertion of the DNA fragment.
- **Genomic libraries** can be made by cloning genomic fragments from one organism into plasmid vectors or into bacteriophage.
- **cDNA libraries** can be generated to represent the mRNA molecules expressed in a cell at a given point.
- **Transfection** of eukaryotic hosts can be achieved through various methods using **electroporation, gene guns, microinjection, shuttle vectors**, and **viral vectors**.

Multiple Choice

Exercise:

Problem:

Which of the following is required for repairing the phosphodiester backbone of DNA during molecular cloning?

- a. cDNA
- b. reverse transcriptase
- c. restriction enzymes
- d. DNA ligase

Solution:

D

Exercise:

Problem:

All of the following are processes used to introduce DNA molecules into bacterial cells *except*:

- a. transformation
 - b. transduction
 - c. transcription
 - d. conjugation
-

Solution:

C

Exercise:

Problem:

The enzyme that uses RNA as a template to produce a DNA copy is called:

- a. a restriction enzyme
 - b. DNA ligase
 - c. reverse transcriptase
 - d. DNA polymerase
-

Solution:

C

Exercise:

Problem: In blue-white screening, what do blue colonies represent?

- a. cells that have not taken up the plasmid vector
 - b. cells with recombinant plasmids containing a new insert
 - c. cells containing empty plasmid vectors
 - d. cells with a non-functional *lacZ* gene
-

Solution:

C

Exercise:

Problem: The T_i plasmid is used for introducing genes into:

- a. animal cells
 - b. plant cells
 - c. bacteriophages
 - d. *E. coli* cells
-

Solution:

B

True/False

Exercise:

Problem: Recombination is a process not usually observed in nature.

Solution:

false

Exercise:

Problem:

It is generally easier to introduce recombinant DNA into prokaryotic cells than into eukaryotic cells.

Solution:

true

Fill in the Blank

Exercise:

Problem:

The process of introducing DNA molecules into eukaryotic cells is called _____.

Solution:

transfection

Short answer

Exercise:

Problem:

Name three elements incorporated into a plasmid vector for efficient cloning.

Exercise:

Problem:

When would a scientist want to generate a cDNA library instead of a genomic library?

Exercise:

Problem:

What is one advantage of generating a genomic library using phages instead of plasmids?

Critical Thinking

Exercise:

Problem:

Is biotechnology always associated with genetic engineering? Explain your answer.

Exercise:

Problem:

Which is more efficient: blunt-end cloning or sticky-end cloning?

Why?

Visualizing and Characterizing DNA, RNA, and Protein

LEARNING OBJECTIVES

- Explain the use of nucleic acid probes to visualize specific DNA sequences
- Explain the use of gel electrophoresis to separate DNA fragments
- Explain the principle of restriction fragment length polymorphism analysis and its uses
- Compare and contrast Southern and northern blots
- Explain the principles and uses of microarray analysis
- Describe the methods used to separate and visualize protein variants
- Explain the method and uses of polymerase chain reaction and DNA sequencing

The sequence of a DNA molecule can help us identify an organism when compared to known sequences housed in a database. The sequence can also tell us something about the function of a particular part of the DNA, such as whether it encodes a particular protein. Comparing **protein signatures**—the expression levels of specific arrays of proteins—between samples is an important method for evaluating cellular responses to a multitude of environmental factors and stresses. Analysis of protein signatures can reveal the identity of an organism or how a cell is responding during disease.

The DNA and proteins of interest are microscopic and typically mixed in with many other molecules including DNA or proteins irrelevant to our

interests. Many techniques have been developed to isolate and characterize molecules of interest. These methods were originally developed for research purposes, but in many cases they have been simplified to the point that routine clinical use is possible. For example, many pathogens, such as the bacterium *Helicobacter pylori*, which causes stomach ulcers, can be detected using protein-based tests. In addition, an increasing number of highly specific and accurate DNA amplification-based identification assays can now detect pathogens such as antibiotic-resistant enteric bacteria, herpes simplex virus, varicella-zoster virus, and many others.

Molecular Analysis of DNA

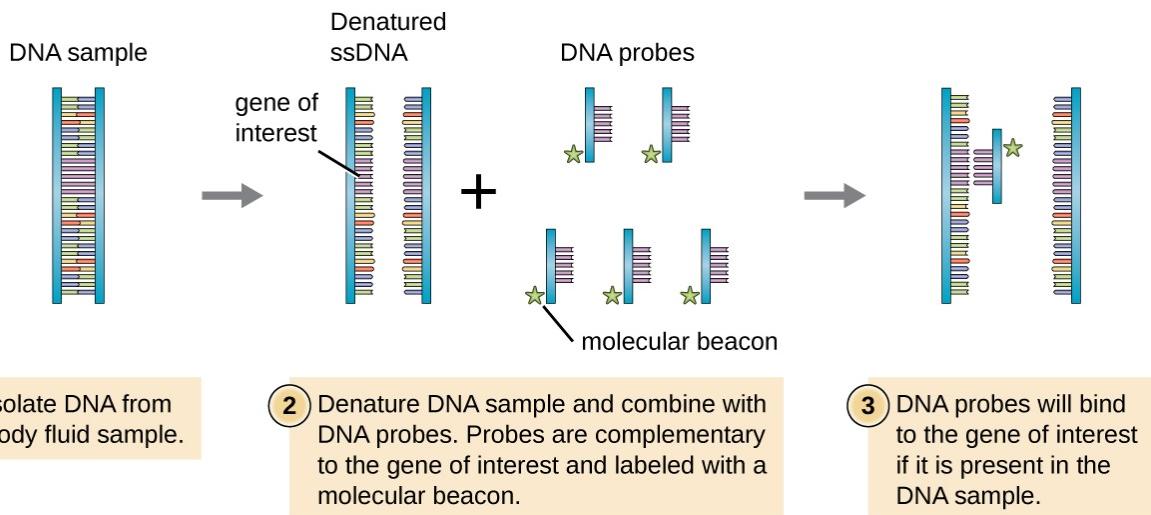
In this subsection, we will outline some of the basic methods used for separating and visualizing specific fragments of DNA that are of interest to a scientist. Some of these methods do not require knowledge of the complete sequence of the DNA molecule. Before the advent of rapid DNA sequencing, these methods were the only ones available to work with DNA, but they still form the basic arsenal of tools used by molecular geneticists to study the body's responses to microbial and other diseases.

Nucleic Acid Probing

DNA molecules are small, and the information contained in their sequence is invisible. How does a researcher isolate a particular stretch of DNA, or having isolated it, determine what organism it is from, what its sequence is, or what its function is? One method to identify the presence of a certain DNA sequence uses artificially constructed pieces of DNA called probes. Probes can be used to identify different bacterial species in the environment and many DNA probes are now available to detect pathogens clinically. For example, DNA probes are used to detect the vaginal pathogens *Candida albicans*, *Gardnerella vaginalis*, and *Trichomonas vaginalis*.

To screen a genomic library for a particular gene or sequence of interest, researchers must know something about that gene. If researchers have a portion of the sequence of DNA for the gene of interest, they can design a

DNA probe, a single-stranded DNA fragment that is complementary to part of the gene of interest and different from other DNA sequences in the sample. The DNA probe may be synthesized chemically by commercial laboratories, or it may be created by cloning, isolating, and denaturing a DNA fragment from a living organism. In either case, the DNA probe must be labeled with a molecular tag or beacon, such as a radioactive phosphorus atom (as is used for **autoradiography**) or a fluorescent dye (as is used in fluorescent *in situ* hybridization, or FISH), so that the probe and the DNA it binds to can be seen ([\[link\]](#)). The DNA sample being probed must also be denatured to make it single-stranded so that the single-stranded DNA probe can anneal to the single-stranded DNA sample at locations where their sequences are complementary. While these techniques are valuable for diagnosis, their direct use on sputum and other bodily samples may be problematic due to the complex nature of these samples. DNA often must first be isolated from bodily samples through chemical extraction methods before a DNA probe can be used to identify pathogens.



DNA probes can be used to confirm the presence of a suspected pathogen in patient samples. This diagram illustrates how a DNA probe can be used to search for a gene of interest associated with the suspected pathogen.

Note:**Part 2**

The mild, flu-like symptoms that Kayla is experiencing could be caused by any number of infectious agents. In addition, several non-infectious autoimmune conditions, such as multiple sclerosis, systemic lupus erythematosus (SLE), and amyotrophic lateral sclerosis (ALS), also have symptoms that are consistent with Kayla's early symptoms. However, over the course of several weeks, Kayla's symptoms worsened. She began to experience joint pain in her knees, heart palpitations, and a strange limpness in her facial muscles. In addition, she suffered from a stiff neck and painful headaches. Reluctantly, she decided it was time to seek medical attention.

- Do Kayla's new symptoms provide any clues as to what type of infection or other medical condition she may have?
- What tests or tools might a health-care provider use to pinpoint the pathogen causing Kayla's symptoms?

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

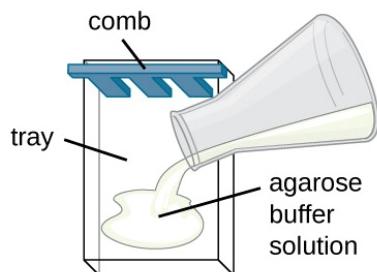
Agarose Gel Electrophoresis

There are a number of situations in which a researcher might want to physically separate a collection of DNA fragments of different sizes. A researcher may also digest a DNA sample with a restriction enzyme to form fragments. The resulting size and fragment distribution pattern can often yield useful information about the sequence of DNA bases that can be used, much like a bar-code scan, to identify the individual or species to which the DNA belongs.

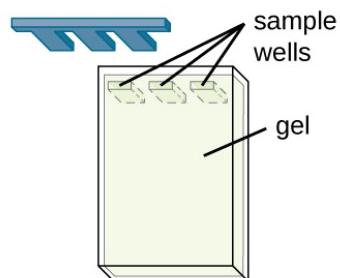
Gel electrophoresis is a technique commonly used to separate biological molecules based on size and biochemical characteristics, such as charge and polarity. **Agarose gel electrophoresis** is widely used to separate DNA (or RNA) of varying sizes that may be generated by restriction enzyme digestion or by other means, such as the PCR ([\[link\]](#)).

Due to its negatively charged backbone, DNA is strongly attracted to a positive electrode. In agarose gel electrophoresis, the gel is oriented horizontally in a buffer solution. Samples are loaded into sample wells on the side of the gel closest to the negative electrode, then drawn through the molecular sieve of the agarose matrix toward the positive electrode. The agarose matrix impedes the movement of larger molecules through the gel, whereas smaller molecules pass through more readily. Thus, the distance of migration is inversely correlated to the size of the DNA fragment, with smaller fragments traveling a longer distance through the gel. Sizes of DNA fragments within a sample can be estimated by comparison to fragments of known size in a DNA ladder also run on the same gel. To separate very large DNA fragments, such as chromosomes or viral genomes, agarose gel electrophoresis can be modified by periodically alternating the orientation of the electric field during pulsed-field gel electrophoresis (PFGE). In PFGE, smaller fragments can reorient themselves and migrate slightly faster than larger fragments and this technique can thus serve to separate very large fragments that would otherwise travel together during standard agarose gel electrophoresis. In any of these electrophoresis techniques, the locations of the DNA or RNA fragments in the gel can be detected by various methods. One common method is adding ethidium bromide, a stain that inserts into the nucleic acids at non-specific locations and can be visualized when exposed to ultraviolet light. Other stains that are safer than ethidium bromide, a potential carcinogen, are now available.

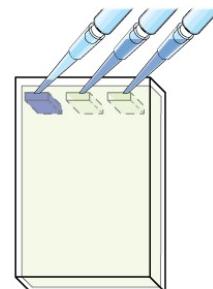
1 An agarose and buffer solution is poured into a plastic tray. A comb is placed into the tray on one end.



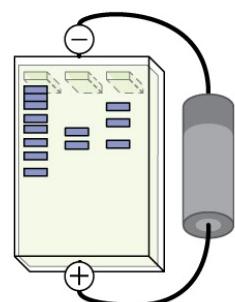
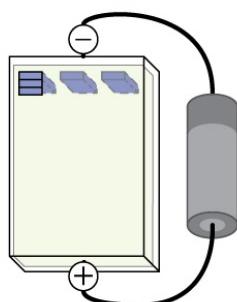
2 The agarose polymerizes into a gel as it cools. The comb is removed from the gel to form wells for samples.



3 DNA samples colored with a tracking dye are pipetted into the wells.

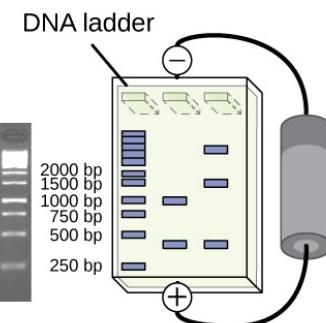


4 The tray is placed into a chamber that generates electric current through the gel. The negative electrode is placed on the side nearest the samples. The positive electrode is placed on the other side.

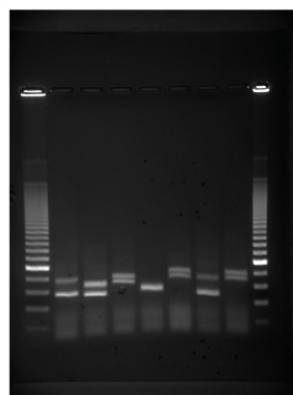


(a)

5 DNA has a negative charge and will be drawn to the positive electrode. Smaller DNA molecules will be able to travel faster through the gel.



(b)



(c)

(a) The process of agarose gel electrophoresis. (b) A researcher loading samples into a gel. (c) This photograph shows a completed electrophoresis run on an agarose gel. The DNA ladder is located in lanes 1 and 9. Seven samples are located in lanes 2 through 8. The gel was stained with ethidium bromide and photographed under ultraviolet light. (credit a: modification of work by Magnus Manske; credit b: modification of work by U.S. Department of Agriculture; credit c: modification of work by James Jacob)

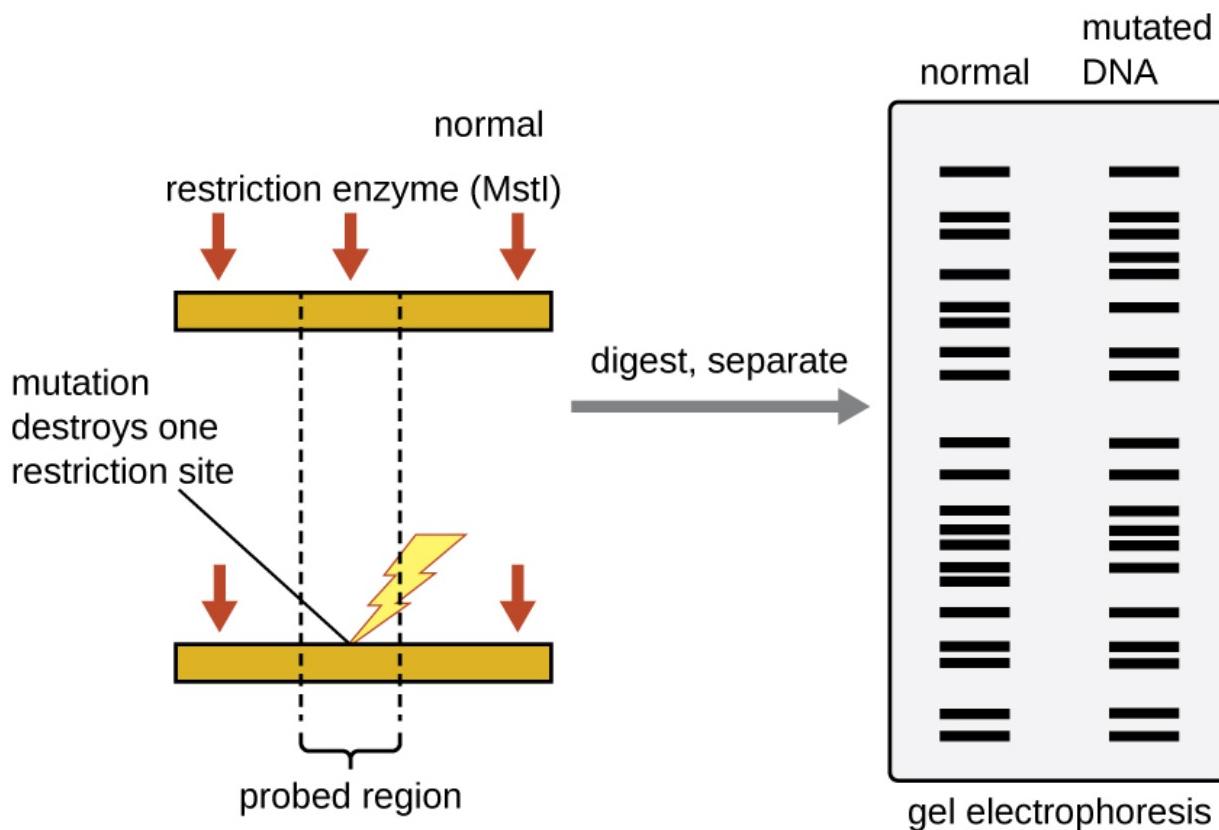
Restriction Fragment Length Polymorphism (RFLP) Analysis

Restriction enzyme recognition sites are short (only a few nucleotides long), sequence-specific palindromes, and may be found throughout the genome. Thus, differences in DNA sequences in the genomes of individuals will lead to differences in distribution of restriction-enzyme recognition sites that can be visualized as distinct banding patterns on a gel after agarose gel electrophoresis. **Restriction fragment length polymorphism (RFLP)** analysis compares DNA banding patterns of different DNA samples after restriction digestion ([\[link\]](#)).

RFLP analysis has many practical applications in both medicine and forensic science. For example, epidemiologists use RFLP analysis to track and identify the source of specific microorganisms implicated in outbreaks of food poisoning or certain infectious diseases. RFLP analysis can also be used on human DNA to determine inheritance patterns of chromosomes with variant genes, including those associated with heritable diseases or to establish paternity.

Forensic scientists use RFLP analysis as a form of DNA fingerprinting, which is useful for analyzing DNA obtained from crime scenes, suspects, and victims. DNA samples are collected, the numbers of copies of the sample DNA molecules are increased using PCR, and then subjected to restriction enzyme digestion and agarose gel electrophoresis to generate specific banding patterns. By comparing the banding patterns of samples

collected from the crime scene against those collected from suspects or victims, investigators can definitively determine whether DNA evidence collected at the scene was left behind by suspects or victims.

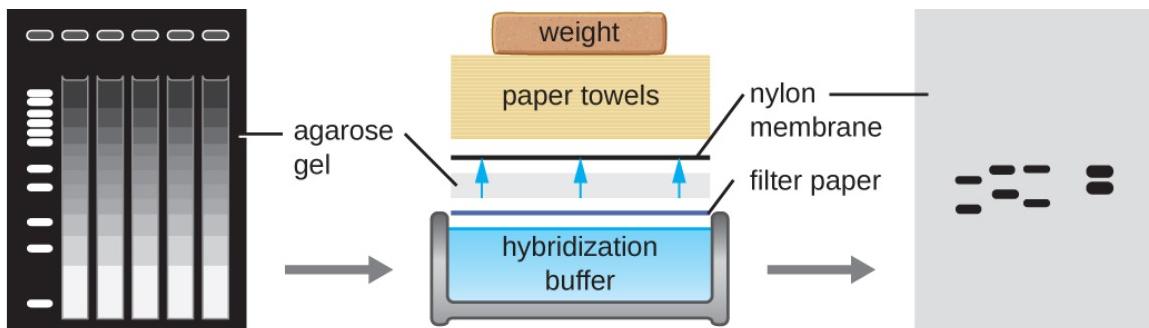


RFLP analysis can be used to differentiate DNA sequences. In this example, a normal chromosome is digested into two fragments, whereas digestion of a mutated chromosome produces only one fragment. The small red arrows pointing to the two different chromosome segments show the locations of the restriction enzyme recognition sites. After digestion and agarose gel electrophoresis, the banding patterns reflect the change by showing the loss of two shorter bands and the gain of a longer band. (credit: modification of work by National Center for Biotechnology Information)

Southern Blots and Modifications

Several molecular techniques capitalize on sequence complementarity and hybridization between nucleic acids of a sample and DNA probes.

Typically, probing nucleic-acid samples within a gel is unsuccessful because as the DNA probe soaks into a gel, the sample nucleic acids within the gel diffuse out. Thus, blotting techniques are commonly used to transfer nucleic acids to a thin, positively charged membrane made of nitrocellulose or nylon. In the **Southern blot** technique, developed by Sir Edwin Southern in 1975, DNA fragments within a sample are first separated by agarose gel electrophoresis and then transferred to a membrane through capillary action ([\[link\]](#)). The DNA fragments that bind to the surface of the membrane are then exposed to a specific single-stranded DNA probe labeled with a radioactive or fluorescent molecular beacon to aid in detection. Southern blots may be used to detect the presence of certain DNA sequences in a given DNA sample. Once the target DNA within the membrane is visualized, researchers can cut out the portion of the membrane containing the fragment to recover the DNA fragment of interest.



- 1 Electrophoresis is used to separate DNA fragments by size. There can be so many fragments that they appear as a smear on the gel.
- 2 The DNA is transferred from the agarose gel to a nylon membrane. The buffer moves from the wet sponge below up through the gel and the membrane into the paper towels; it carries the DNA fragments with it until they become caught in the membrane.
- 3 The membrane is bathed in a solution containing a probe, a short piece of DNA complementary to the sequence of interest. The probe is labeled or tagged with a fluorescent dye so that the location of DNA fragments to which it hybridizes can be visualized.

In the Southern blot technique, DNA fragments are first separated by agarose gel electrophoresis, then transferred by capillary action to a nylon membrane, which is then soaked with a DNA probe tagged with a molecular beacon for easy visualization.

Variations of the Southern blot—the dot blot, slot blot, and the spot blot—do not involve electrophoresis, but instead concentrate DNA from a sample into a small location on a membrane. After hybridization with a DNA probe, the signal intensity detected is measured, allowing the researcher to estimate the amount of target DNA present within the sample.

A colony blot is another variation of the Southern blot in which colonies representing different clones in a genomic library are transferred to a membrane by pressing the membrane onto the culture plate. The cells on the membrane are lysed and the membrane can then be probed to determine which colonies within a genomic library harbor the target gene. Because the

colonies on the plate are still growing, the cells of interest can be isolated from the plate.

In the **northern blot**, another variation of the Southern blot, RNA (not DNA) is immobilized on the membrane and probed. Northern blots are typically used to detect the amount of mRNA made through gene expression within a tissue or organism sample.

Microarray Analysis

Another technique that capitalizes on the hybridization between complementary nucleic acid sequences is called **microarray analysis**. Microarray analysis is useful for the comparison of gene-expression patterns between different cell types—for example, cells infected with a virus versus uninfected cells, or cancerous cells versus healthy cells ([\[link\]](#)).

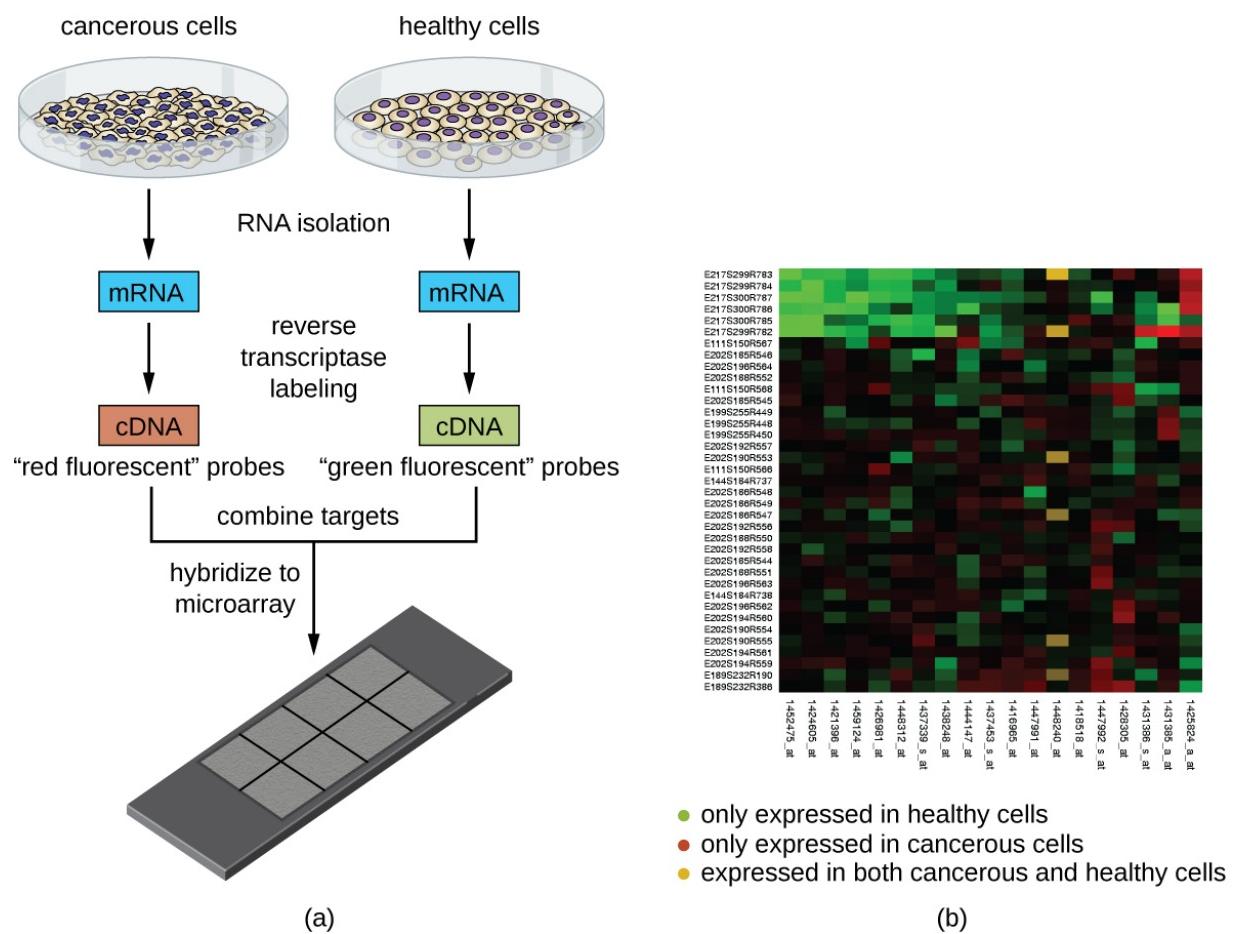
Typically, DNA or cDNA from an experimental sample is deposited on a glass slide alongside known DNA sequences. Each slide can hold more than 30,000 different DNA fragment types. Distinct DNA fragments (encompassing an organism's entire genomic library) or cDNA fragments (corresponding to an organism's full complement of expressed genes) can be individually spotted on a glass slide.

Once deposited on the slide, genomic DNA or mRNA can be isolated from the two samples for comparison. If mRNA is isolated, it is reverse-transcribed to cDNA using reverse transcriptase. Then the two samples of genomic DNA or cDNA are labeled with different fluorescent dyes (typically red and green). The labeled genomic DNA samples are then combined in equal amounts, added to the microarray chip, and allowed to hybridize to complementary spots on the microarray.

Hybridization of sample genomic DNA molecules can be monitored by measuring the intensity of fluorescence at particular spots on the microarray. Differences in the amount of hybridization between the samples can be readily observed. If only one sample's nucleic acids hybridize to a particular spot on the microarray, then that spot will appear either green or

red. However, if both samples' nucleic acids hybridize, then the spot will appear yellow due to the combination of the red and green dyes.

Although microarray technology allows for a holistic comparison between two samples in a short time, it requires sophisticated (and expensive) detection equipment and analysis software. Because of the expense, this technology is typically limited to research settings. Researchers have used microarray analysis to study how gene expression is affected in organisms that are infected by bacteria or viruses or subjected to certain chemical treatments.



(a) The steps in microarray analysis are illustrated. Here, gene expression patterns are compared between cancerous cells and healthy cells. (b) Microarray information can be expressed as a heat map. Genes are shown on the left side; different samples are shown across

the bottom. Genes expressed only in cancer cells are shown in varying shades of red; genes expressed only in normal cells are shown in varying shades of green. Genes that are expressed in both cancerous and normal cells are shown in yellow.

Note:



Explore [microchip technology](#) at this interactive website.

Note:

- What does a DNA probe consist of?
- Why is a Southern blot used after gel electrophoresis of a DNA digest?

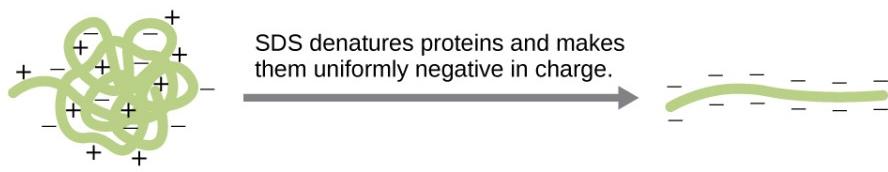
Molecular Analysis of Proteins

In many cases it may not be desirable or possible to study DNA or RNA directly. Proteins can provide species-specific information for identification as well as important information about how and whether a cell or tissue is

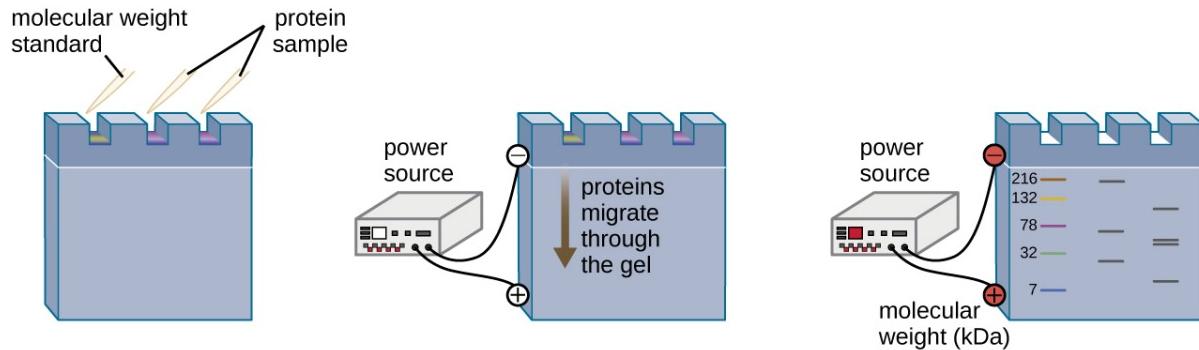
responding to the presence of a pathogenic microorganism. Various proteins require different methods for isolation and characterization.

Polyacrylamide Gel Electrophoresis

A variation of gel electrophoresis, called **polyacrylamide gel electrophoresis (PAGE)**, is commonly used for separating proteins. In PAGE, the gel matrix is finer and composed of polyacrylamide instead of agarose. Additionally, PAGE is typically performed using a vertical gel apparatus ([\[link\]](#)). Because of the varying charges associated with amino acid side chains, PAGE can be used to separate intact proteins based on their net charges. Alternatively, proteins can be denatured and coated with a negatively charged detergent called sodium dodecyl sulfate (SDS), masking the native charges and allowing separation based on size only. PAGE can be further modified to separate proteins based on two characteristics, such as their charges at various pHs as well as their size, through the use of two-dimensional PAGE. In any of these cases, following electrophoresis, proteins are visualized through staining, commonly with either Coomassie blue or a silver stain.



(a)

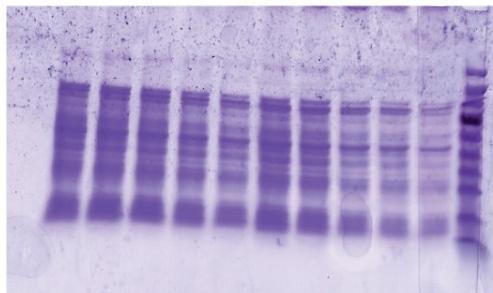


Samples are loaded into the wells.

Small proteins travel through the gel faster than large proteins.

The molecular weight standard includes fragments of known size and is used to estimate the size of sample proteins.

(b)



(c)

(a) SDS is a detergent that denatures proteins and masks their native charges, making them uniformly negatively charged. (b) The process of SDS-PAGE is illustrated in these steps. (c) A photograph of an SDS-PAGE gel shows Coomassie stained bands where proteins of different size have migrated along the gel in response to the applied voltage. A size standard lane is visible on the right side of the gel.
 (credit b: modification of work by “GeneEd”/YouTube)

Note:

- On what basis are proteins separated in SDS-PAGE?

Note:**Part 3**

When Kayla described her symptoms, her physician at first suspected bacterial meningitis, which is consistent with her headaches and stiff neck. However, she soon ruled this out as a possibility because meningitis typically progresses more quickly than what Kayla was experiencing. Many of her symptoms still paralleled those of amyotrophic lateral sclerosis (ALS) and systemic lupus erythematosus (SLE), and the physician also considered Lyme disease a possibility given how much time Kayla spends in the woods. Kayla did not recall any recent tick bites (the typical means by which Lyme disease is transmitted) and she did not have the typical bull's-eye rash associated with Lyme disease ([\[link\]](#)). However, 20–30% of patients with Lyme disease never develop this rash, so the physician did not want to rule it out.

Kayla's doctor ordered an MRI of her brain, a complete blood count to test for anemia, blood tests assessing liver and kidney function, and additional tests to confirm or rule out SLE or Lyme disease. Her test results were inconsistent with both SLE and ALS, and the result of the test looking for Lyme disease antibodies was “equivocal,” meaning inconclusive. Having ruled out ALS and SLE, Kayla's doctor decided to run additional tests for Lyme disease.

- Why would Kayla's doctor still suspect Lyme disease even if the test results did not detect Lyme antibodies in the blood?
- What type of molecular test might be used for the detection of blood antibodies to Lyme disease?



A bulls-eye rash is one of the common symptoms of Lyme diseases, but up to 30% of infected individuals never develop a rash. (credit: Centers for Disease Control and Prevention)

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

Amplification-Based DNA Analysis Methods

Various methods can be used for obtaining sequences of DNA, which are useful for studying disease-causing organisms. With the advent of rapid sequencing technology, our knowledge base of the entire genomes of pathogenic organisms has grown phenomenally. We start with a description of the polymerase chain reaction, which is not a sequencing method but has allowed researchers and clinicians to obtain the large quantities of DNA

needed for sequencing and other studies. The polymerase chain reaction eliminates the dependence we once had on cells to make multiple copies of DNA, achieving the same result through relatively simple reactions outside the cell.

Polymerase Chain Reaction (PCR)

Most methods of DNA analysis, such as restriction enzyme digestion and agarose gel electrophoresis, or DNA sequencing require large amounts of a specific DNA fragment. In the past, large amounts of DNA were produced by growing the host cells of a genomic library. However, libraries take time and effort to prepare and DNA samples of interest often come in minute quantities. The **polymerase chain reaction (PCR)** permits rapid amplification in the number of copies of specific DNA sequences for further analysis ([\[link\]](#)). One of the most powerful techniques in molecular biology, PCR was developed in 1983 by Kary Mullis while at Cetus Corporation. PCR has specific applications in research, forensic, and clinical laboratories, including:

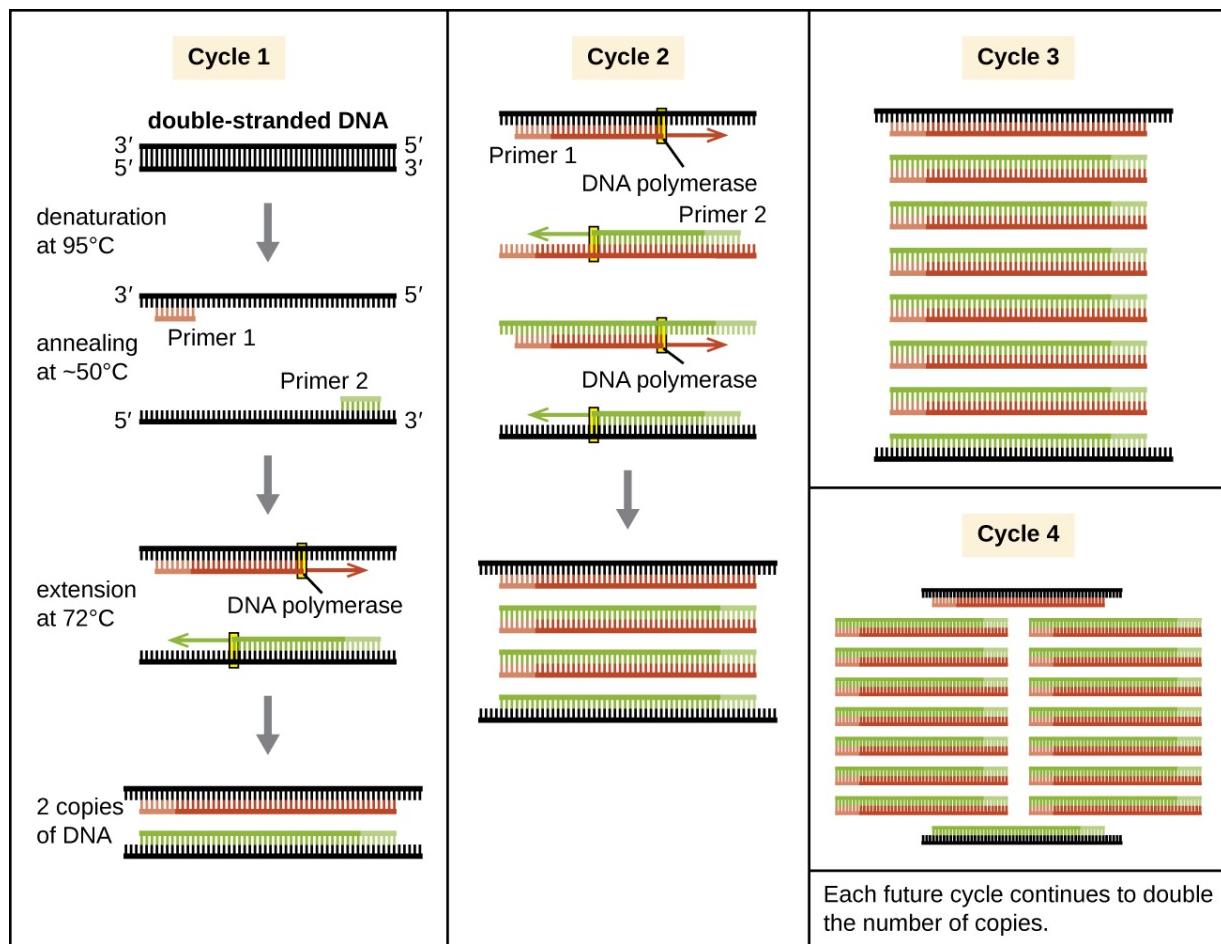
- determining the sequence of nucleotides in a specific region of DNA
- amplifying a target region of DNA for cloning into a plasmid vector
- identifying the source of a DNA sample left at a crime scene
- analyzing samples to determine paternity
- comparing samples of ancient DNA with modern organisms
- determining the presence of difficult to culture, or unculturable, microorganisms in humans or environmental samples

PCR is an *in vitro* laboratory technique that takes advantage of the natural process of DNA replication. The heat-stable DNA polymerase enzymes used in PCR are derived from hyperthermophilic prokaryotes. *Taq DNA polymerase*, commonly used in PCR, is derived from the *Thermus aquaticus* bacterium isolated from a hot spring in Yellowstone National Park. DNA replication requires the use of primers for the initiation of replication to have free 3'-hydroxyl groups available for the addition of nucleotides by DNA polymerase. However, while primers composed of RNA are normally used in cells, DNA primers are used for PCR. **DNA**

primers are preferable due to their stability, and DNA primers with known sequences targeting a specific DNA region can be chemically synthesized commercially. These DNA primers are functionally similar to the DNA probes used for the various hybridization techniques described earlier, binding to specific targets due to complementarity between the target DNA sequence and the primer.

PCR occurs over multiple cycles, each containing three steps: denaturation, annealing, and extension. Machines called thermal cyclers are used for PCR; these machines can be programmed to automatically cycle through the temperatures required at each step ([\[link\]](#)). First, double-stranded template DNA containing the target sequence is denatured at approximately 95 °C. The high temperature required to physically (rather than enzymatically) separate the DNA strands is the reason the heat-stable DNA polymerase is required. Next, the temperature is lowered to approximately 50 °C. This allows the DNA primers complementary to the ends of the target sequence to anneal (stick) to the template strands, with one primer annealing to each strand. Finally, the temperature is raised to 72 °C, the optimal temperature for the activity of the heat-stable DNA polymerase, allowing for the addition of nucleotides to the primer using the single-stranded target as a template. Each cycle doubles the number of double-stranded target DNA copies. Typically, PCR protocols include 25–40 cycles, allowing for the amplification of a single target sequence by tens of millions to over a trillion.

Natural DNA replication is designed to copy the entire genome, and initiates at one or more origin sites. Primers are constructed during replication, not before, and do not consist of a few specific sequences. PCR targets specific regions of a DNA sample using sequence-specific primers. In recent years, a variety of isothermal PCR amplification methods that circumvent the need for thermal cycling have been developed, taking advantage of accessory proteins that aid in the DNA replication process. As the development of these methods continues and their use becomes more widespread in research, forensic, and clinical labs, thermal cyclers may become obsolete.



■ parent/original DNA
 ■ DNA primer attaching to 3'-5' strand
 ■ DNA primer attaching to 5'-3' strand

The polymerase chain reaction (PCR) is used to produce many copies of a specific sequence of DNA.

Note:



Deepen your understanding of the polymerase chain reaction by viewing this [animation](#) and working through an [interactive](#) exercise.

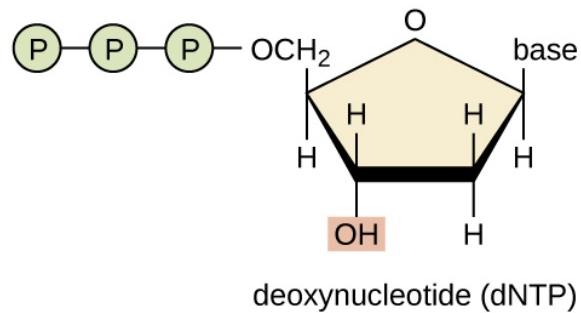
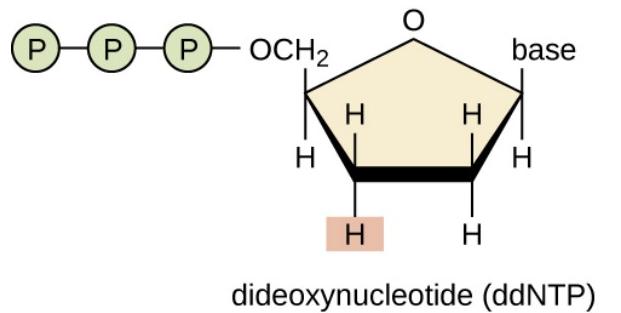
PCR Variations

Several later modifications to PCR further increase the utility of this technique. **Reverse transcriptase PCR (RT-PCR)** is used for obtaining DNA copies of a specific mRNA molecule. RT-PCR begins with the use of the reverse transcriptase enzyme to convert mRNA molecules into cDNA. That cDNA is then used as a template for traditional PCR amplification. RT-PCR can detect whether a specific gene has been expressed in a sample. Another recent application of PCR is **real-time PCR**, also known as **quantitative PCR (qPCR)**. Standard PCR and RT-PCR protocols are not quantitative because any one of the reagents may become limiting before all of the cycles within the protocol are complete, and samples are only analyzed at the end. Because it is not possible to determine when in the PCR or RT-PCR protocol a given reagent has become limiting, it is not possible to know how many cycles were completed prior to this point, and thus it is not possible to determine how many original template molecules were present in the sample at the start of PCR. In qPCR, however, the use of fluorescence allows one to monitor the increase in a double-stranded template during a PCR reaction as it occurs. These kinetics data can then be used to quantify the amount of the original target sequence. The use of qPCR in recent years has further expanded the capabilities of PCR, allowing researchers to determine the number of DNA copies, and sometimes organisms, present in a sample. In clinical settings, qRT-PCR is used to determine viral load in HIV-positive patients to evaluate the effectiveness of their therapy.

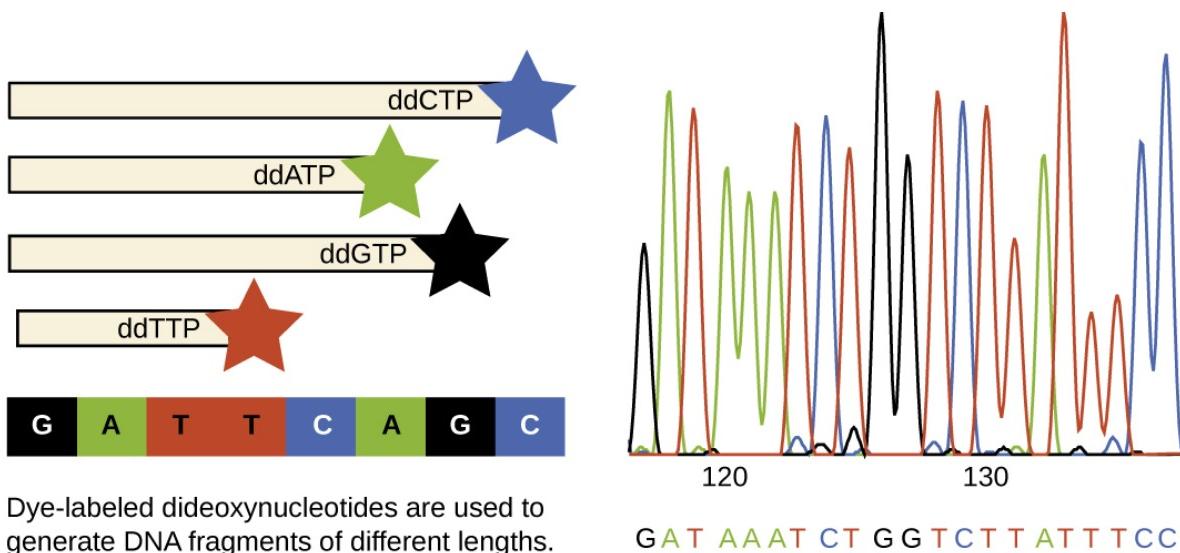
DNA Sequencing

A basic sequencing technique is the **chain termination method**, also known as the **dideoxy method** or the **Sanger DNA sequencing method**, developed by Frederick Sanger in 1972. The chain termination method involves DNA replication of a single-stranded template with the use of a DNA primer to initiate synthesis of a complementary strand, DNA polymerase, a mix of the four regular deoxynucleotide (dNTP) monomers, and a small proportion of dideoxynucleotides (ddNTPs), each labeled with a molecular beacon. The ddNTPs are monomers missing a hydroxyl group ($-OH$) at the site at which another nucleotide usually attaches to form a chain ([\[link\]](#)). Every time a ddNTP is randomly incorporated into the growing complementary strand, it terminates the process of DNA replication for that particular strand. This results in multiple short strands of replicated DNA that are each terminated at a different point during replication. When the reaction mixture is subjected to gel electrophoresis, the multiple newly replicated DNA strands form a ladder of differing sizes. Because the ddNTPs are labeled, each band on the gel reflects the size of the DNA strand when the ddNTP terminated the reaction.

In Sanger's day, four reactions were set up for each DNA molecule being sequenced, each reaction containing only one of the four possible ddNTPs. Each ddNTP was labeled with a radioactive phosphorus molecule. The products of the four reactions were then run in separate lanes side by side on long, narrow PAGE gels, and the bands of varying lengths were detected by autoradiography. Today, this process has been simplified with the use of ddNTPs, each labeled with a different colored fluorescent dye or fluorochrome ([\[link\]](#)), in one sequencing reaction containing all four possible ddNTPs for each DNA molecule being sequenced ([\[link\]](#)). These fluorochromes are detected by fluorescence spectroscopy. Determining the fluorescence color of each band as it passes by the detector produces the nucleotide sequence of the template strand.



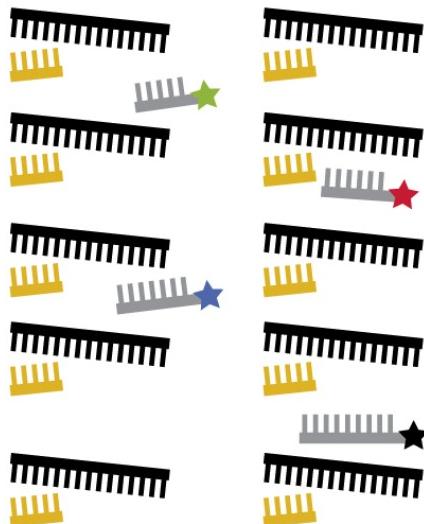
A dideoxynucleotide is similar in structure to a deoxynucleotide, but is missing the 3' hydroxyl group (indicated by the shaded box). When a dideoxynucleotide is incorporated into a DNA strand, DNA synthesis stops.



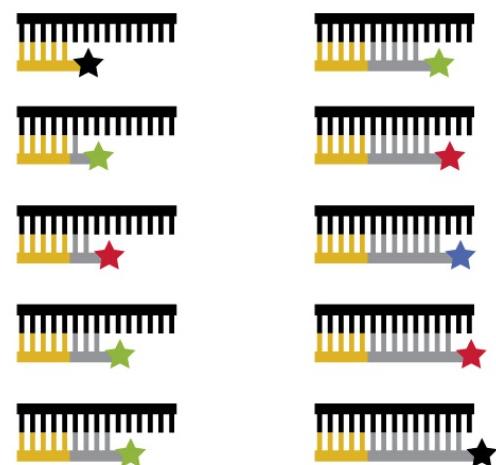
Dye-labeled dideoxynucleotides are used to generate DNA fragments of different lengths.

Frederick Sanger's dideoxy chain termination method is illustrated, using ddNTPs tagged with fluorochromes. Using ddNTPs, a mixture of DNA fragments of every possible size, varying in length by only one nucleotide, can be generated. The DNA is separated on the basis of size and each band can be detected with a fluorescence detector.

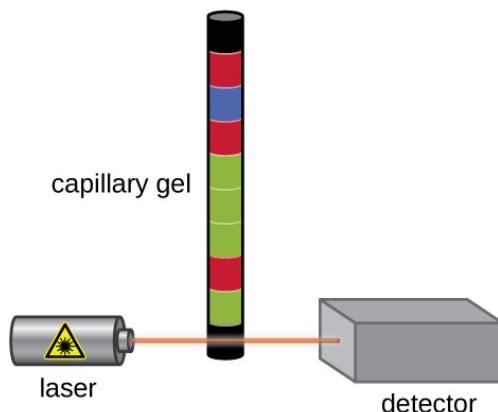
① The following are added to the PCR reaction tube: DNA template, primers, DNA polymerase, dNTPs, and fluorescently labeled ddNTPs.



② At each location either a dNTP is added and elongation continues, or a ddNTP is added and elongation stops. This process results in fragments of all sizes, each with a different fluorescently labeled end nucleotide.



③ The fragments are run through a capillary gel.



④ A computer identifies each band as it passes by a laser.



This diagram summarizes the Sanger sequencing method using fluorochrome-labeled ddNTPs and capillary gel electrophoresis.

Since 2005, automated sequencing techniques used by laboratories fall under the umbrella of **next generation sequencing**, which is a group of automated techniques used for rapid DNA sequencing. These methods have

revolutionized the field of molecular genetics because the low-cost sequencers can generate sequences of hundreds of thousands or millions of short fragments (25 to 600 base pairs) just in one day. Although several variants of next generation sequencing technologies are made by different companies (for example, 454 Life Sciences' pyrosequencing and Illumina's Solexa technology), they all allow millions of bases to be sequenced quickly, making the sequencing of entire genomes relatively easy, inexpensive, and commonplace. In **454 sequencing (pyrosequencing)**, for example, a DNA sample is fragmented into 400–600-bp single-strand fragments, modified with the addition of DNA adapters to both ends of each fragment. Each DNA fragment is then immobilized on a bead and amplified by PCR, using primers designed to anneal to the adapters, creating a bead containing many copies of that DNA fragment. Each bead is then put into a separate well containing sequencing enzymes. To the well, each of the four nucleotides is added one after the other; when each one is incorporated, pyrophosphate is released as a byproduct of polymerization, emitting a small flash of light that is recorded by a detector. This provides the order of nucleotides incorporated as a new strand of DNA is made and is an example of synthesis sequencing. Next generation sequencers use sophisticated software to get through the cumbersome process of putting all the fragments in order. Overall, these technologies continue to advance rapidly, decreasing the cost of sequencing and increasing the availability of sequence data from a wide variety of organisms quickly.

The National Center for Biotechnology Information houses a widely used genetic sequence database called GenBank where researchers deposit genetic information for public use. Upon publication of sequence data, researchers upload it to GenBank, giving other researchers access to the information. The collaboration allows researchers to compare newly discovered or unknown sample sequence information with the vast array of sequence data that already exists.

Note:



View an [animation](#) about 454 sequencing to deepen your understanding of this method.

Note:

Using a NAAT to Diagnose a *C. difficile* Infection

Javier, an 80-year-old patient with a history of heart disease, recently returned home from the hospital after undergoing an angioplasty procedure to insert a stent into a cardiac artery. To minimize the possibility of infection, Javier was administered intravenous broad-spectrum antibiotics during and shortly after his procedure. He was released four days after the procedure, but a week later, he began to experience mild abdominal cramping and watery diarrhea several times a day. He lost his appetite, became severely dehydrated, and developed a fever. He also noticed blood in his stool. Javier's wife called the physician, who instructed her to take him to the emergency room immediately.

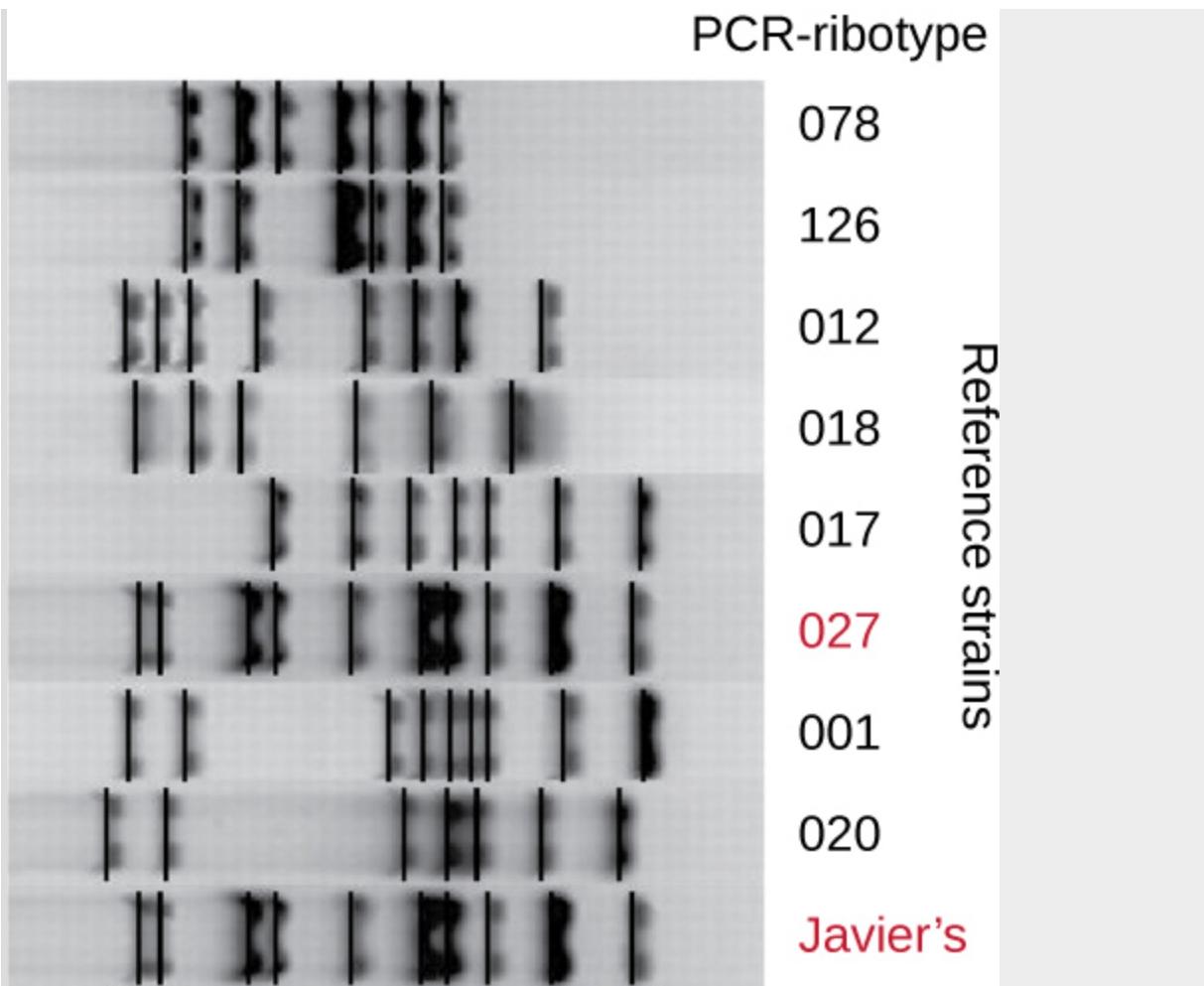
The hospital staff ran several tests and found that Javier's kidney creatinine levels were elevated compared with the levels in his blood, indicating that his kidneys were not functioning well. Javier's symptoms suggested a possible infection with *Clostridium difficile*, a bacterium that is resistant to many antibiotics. The hospital collected and cultured a stool sample to look for the production of toxins A and B by *C. difficile*, but the results came back negative. However, the negative results were not enough to rule out a *C. difficile* infection because culturing of *C. difficile* and detection of its characteristic toxins can be difficult, particularly in some types of samples. To be safe, they proceeded with a diagnostic nucleic acid amplification test (NAAT). Currently NAATs are the clinical diagnostician's gold standard for detecting the genetic material of a pathogen. In Javier's case, qPCR

was used to look for the gene encoding *C. difficile* toxin B (*tcdB*). When the qPCR analysis came back positive, the attending physician concluded that Javier was indeed suffering from a *C. difficile* infection and immediately prescribed the antibiotic vancomycin, to be administered intravenously. The antibiotic cleared the infection and Javier made a full recovery.

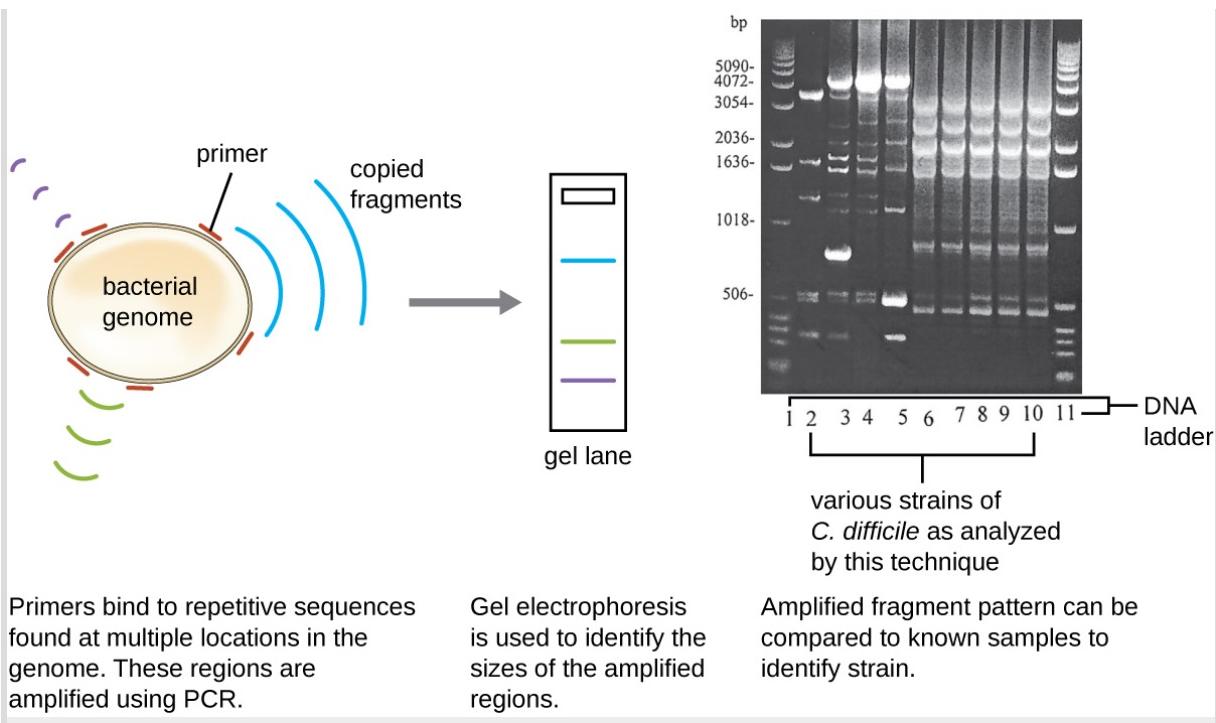
Because infections with *C. difficile* were becoming widespread in Javier's community, his sample was further analyzed to see whether the specific strain of *C. difficile* could be identified. Javier's stool sample was subjected to ribotyping and repetitive sequence-based PCR (rep-PCR) analysis. In ribotyping, a short sequence of DNA between the 16S rRNA and 23S rRNA genes is amplified and subjected to restriction digestion ([\[link\]](#)). This sequence varies between strains of *C. difficile*, so restriction enzymes will cut in different places. In rep-PCR, DNA primers designed to bind to short sequences commonly found repeated within the *C. difficile* genome were used for PCR. Following restriction digestion, agarose gel electrophoresis was performed in both types of analysis to examine the banding patterns that resulted from each procedure ([\[link\]](#)). Rep-PCR can be used to further subtype various ribotypes, increasing resolution for detecting differences between strains. The ribotype of the strain infecting Javier was found to be ribotype 27, a strain known for its increased virulence, resistance to antibiotics, and increased prevalence in the United States, Canada, Japan, and Europe.[\[footnote\]](#)

Patrizia Spigaglia, Fabrizio Barbanti, Anna Maria Dionisi, and Paola Mastrantonio. “*Clostridium difficile* Isolates Resistant to Fluoroquinolones in Italy: Emergence of PCR Ribotype 018.” *Journal of Clinical Microbiology* 48 no. 8 (2010): 2892–2896.

- How do banding patterns differ between strains of *C. difficile*?
- Why do you think laboratory tests were unable to detect toxin production directly?



A gel showing PCR products of various *Clostridium difficile* strains. Javier's sample is shown at the bottom; note that it matches ribotype 27 in the reference set.
(credit: modification of work by American Society for Microbiology)



Strains of infectious bacteria, such as *C. difficile*, can be identified by molecular analysis. PCR ribotyping is commonly used to identify particular *C. difficile* strains. Rep-PCR is an alternate molecular technique that is also used to identify particular *C. difficile* strains.

(credit b: modification of work by American Society for Microbiology)

Note:

- How is PCR similar to the natural DNA replication process in cells? How is it different?
- Compare RT-PCR and qPCR in terms of their respective purposes.
- In chain-termination sequencing, how is the identity of each nucleotide in a sequence determined?

Key Concepts and Summary

- Finding a gene of interest within a sample requires the use of a single-stranded **DNA probe** labeled with a molecular beacon (typically radioactivity or fluorescence) that can hybridize with a complementary single-stranded nucleic acid in the sample.
- **Agarose gel electrophoresis** allows for the separation of DNA molecules based on size.
- **Restriction fragment length polymorphism (RFLP)** analysis allows for the visualization by agarose gel electrophoresis of distinct variants of a DNA sequence caused by differences in restriction sites.
- **Southern blot** analysis allows researchers to find a particular DNA sequence within a sample whereas **northern blot** analysis allows researchers to detect a particular mRNA sequence expressed in a sample.
- **Microarray technology** is a nucleic acid hybridization technique that allows for the examination of many thousands of genes at once to find differences in genes or gene expression patterns between two samples of genomic DNA or cDNA,
- **Polyacrylamide gel electrophoresis (PAGE)** allows for the separation of proteins by size, especially if native protein charges are masked through pretreatment with SDS.
- **Polymerase chain reaction** allows for the rapid amplification of a specific DNA sequence. Variations of PCR can be used to detect mRNA expression (**reverse transcriptase PCR**) or to quantify a particular sequence in the original sample (**real-time PCR**).
- Although the development of **Sanger DNA sequencing** was revolutionary, advances in **next generation sequencing** allow for the rapid and inexpensive sequencing of the genomes of many organisms, accelerating the volume of new sequence data.

Multiple Choice

Exercise:

Problem:

Which technique is used to separate protein fragments based on size?

- a. polyacrylamide gel electrophoresis
 - b. Southern blot
 - c. agarose gel electrophoresis
 - d. polymerase chain reaction
-

Solution:

A

Exercise:**Problem:**

Which technique uses restriction enzyme digestion followed by agarose gel electrophoresis to generate a banding pattern for comparison to another sample processed in the same way?

- a. qPCR
 - b. RT-PCR
 - c. RFLP
 - d. 454 sequencing
-

Solution:

C

Exercise:**Problem:**

All of the following techniques involve hybridization between single-stranded nucleic acid molecules *except*:

- a. Southern blot analysis

- b. RFLP analysis
 - c. northern blot analysis
 - d. microarray analysis
-

Solution:

B

Fill in the Blank

Exercise:

Problem:

The _____ blot technique is used to find an RNA fragment within a sample that is complementary to a DNA probe.

Solution:

northern

Exercise:

Problem:

The PCR step during which the double-stranded template molecule becomes single-stranded is called _____.

Solution:

denaturation

Exercise:

Problem:

The sequencing method involving the incorporation of ddNTPs is called _____.

Solution:

Sanger sequencing, dideoxy method, or chain termination method

True/False**Exercise:****Problem:**

In agarose gel electrophoresis, DNA will be attracted to the negative electrode.

Solution:

false

Short answer**Exercise:****Problem:**

Why is it important that a DNA probe be labeled with a molecular beacon?

Exercise:**Problem:**

When separating proteins strictly by size, why is exposure to SDS first required?

Exercise:**Problem:**

Why must the DNA polymerase used during PCR be heat-stable?

Critical Thinking

Exercise:

Problem:

Suppose you are working in a molecular biology laboratory and are having difficulty performing the PCR successfully. You decide to double-check the PCR protocol programmed into the thermal cycler and discover that the annealing temperature was programmed to be 65 °C instead of 50 °C, as you had intended. What effects would this mistake have on the PCR reaction? Refer to [\[link\]](#).

Exercise:

Problem:

What is the advantage of microarray analysis over northern blot analysis in monitoring changes in gene expression?

Exercise:

Problem:

What is the difference between reverse transcriptase PCR (RT-PCR) and real-time quantitative PCR (qPCR)?

Whole Genome Methods and Pharmaceutical Applications of Genetic Engineering

LEARNING OBJECTIVES

- Explain the uses of genome-wide comparative analyses
- Summarize the advantages of genetically engineered pharmaceutical products

Advances in molecular biology have led to the creation of entirely new fields of science. Among these are fields that study aspects of whole genomes, collectively referred to as whole-genome methods. In this section, we'll provide a brief overview of the whole-genome fields of genomics, transcriptomics, and proteomics.

Genomics, Transcriptomics, and Proteomics

The study and comparison of entire genomes, including the complete set of genes and their nucleotide sequence and organization, is called **genomics**. This field has great potential for future medical advances through the study of the human genome as well as the genomes of infectious organisms. Analysis of microbial genomes has contributed to the development of new antibiotics, diagnostic tools, vaccines, medical treatments, and environmental cleanup techniques.

The field of **transcriptomics** is the science of the entire collection of mRNA molecules produced by cells. Scientists compare gene expression patterns between infected and uninfected host cells, gaining important information about the cellular responses to infectious disease. Additionally,

transcriptomics can be used to monitor the gene expression of virulence factors in microorganisms, aiding scientists in better understanding pathogenic processes from this viewpoint.

When genomics and transcriptomics are applied to entire microbial communities, we use the terms **metagenomics** and **metatranscriptomics**, respectively. Metagenomics and metatranscriptomics allow researchers to study genes and gene expression from a collection of multiple species, many of which may not be easily cultured or cultured at all in the laboratory. A DNA microarray (discussed in the previous section) can be used in metagenomics studies.

Another up-and-coming clinical application of genomics and transcriptomics is **pharmacogenomics**, also called **toxicogenomics**, which involves evaluating the effectiveness and safety of drugs on the basis of information from an individual's genomic sequence. Genomic responses to drugs can be studied using experimental animals (such as laboratory rats or mice) or live cells in the laboratory before embarking on studies with humans. Changes in gene expression in the presence of a drug can sometimes be an early indicator of the potential for toxic effects. Personal genome sequence information may someday be used to prescribe medications that will be most effective and least toxic on the basis of the individual patient's genotype.

The study of **proteomics** is an extension of genomics that allows scientists to study the entire complement of proteins in an organism, called the proteome. Even though all cells of a multicellular organism have the same set of genes, cells in various tissues produce different sets of proteins. Thus, the genome is constant, but the proteome varies and is dynamic within an organism. Proteomics may be used to study which proteins are expressed under various conditions within a single cell type or to compare protein expression patterns between different organisms.

The most prominent disease being studied with proteomic approaches is cancer, but this area of study is also being applied to infectious diseases. Research is currently underway to examine the feasibility of using proteomic approaches to diagnose various types of hepatitis, tuberculosis,

and HIV infection, which are rather difficult to diagnose using currently available techniques.[\[footnote\]](#)

E.O. List, D.E. Berryman, B. Bower, L. Sackmann-Sala, E. Gosney, J. Ding, S. Okada, and J.J. Kopchick. “The Use of Proteomics to Study Infectious Diseases.” *Infectious Disorders-Drug Targets* (Formerly *Current Drug Targets-Infectious Disorders*) 8 no. 1 (2008): 31–45.

A recent and developing proteomic analysis relies on identifying proteins called **biomarkers**, whose expression is affected by the disease process. Biomarkers are currently being used to detect various forms of cancer as well as infections caused by pathogens such as *Yersinia pestis* and *Vaccinia virus*.[\[footnote\]](#)

Mohan Natesan, and Robert G. Ulrich. “Protein Microarrays and Biomarkers of Infectious Disease.” *International Journal of Molecular Sciences* 11 no. 12 (2010): 5165–5183.

Other “-omic” sciences related to genomics and proteomics include metabolomics, glycomics, and lipidomics, which focus on the complete set of small-molecule metabolites, sugars, and lipids, respectively, found within a cell. Through these various global approaches, scientists continue to collect, compile, and analyze large amounts of genetic information. This emerging field of **bioinformatics** can be used, among many other applications, for clues to treating diseases and understanding the workings of cells.

Additionally, researchers can use reverse genetics, a technique related to classic mutational analysis, to determine the function of specific genes. Classic methods of studying gene function involved searching for the genes responsible for a given phenotype. Reverse genetics uses the opposite approach, starting with a specific DNA sequence and attempting to determine what phenotype it produces. Alternatively, scientists can attach known genes (called reporter genes) that encode easily observable characteristics to genes of interest, and the location of expression of such genes of interest can be easily monitored. This gives the researcher important information about what the gene product might be doing or where it is located in the organism. Common reporter genes include bacterial *lacZ*, which encodes beta-galactosidase and whose activity can be monitored by

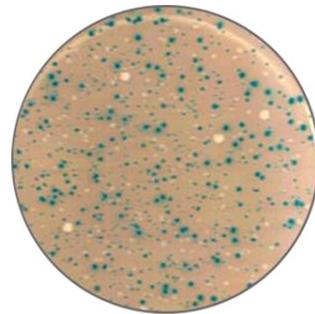
changes in colony color in the presence of X-gal as previously described, and the gene encoding the jellyfish protein green fluorescent protein (GFP) whose activity can be visualized in colonies under ultraviolet light exposure ([\[link\]](#)).



(a)



(b)



(c)

(a) The gene encoding green fluorescence protein is a commonly used reporter gene for monitoring gene expression patterns in organisms. Under ultraviolet light, GFP fluoresces. Here, two mice are expressing GFP, while the middle mouse is not. (b) GFP can be used as a reporter gene in bacteria as well. Here, a plate containing bacterial colonies expressing GFP is shown. (c) Blue-white screening in bacteria is accomplished through the use of the *lacZ* reporter gene, followed by plating of bacteria onto medium containing X-gal. Cleavage of X-gal by the LacZ enzyme results in the formation of blue colonies. (credit a: modification of work by Ingrid Moen, Charlotte Jevne, Jian Wang, Karl-Henning Kalland, Martha Chekenya, Lars A Akslen, Linda Sleire, Per Ø Enger, Rolf K Reed, Anne M Øyan, Linda EB Stuhr; credit b: modification of work by “2.5JIGEN.com”/Flickr; credit c: modification of work by American Society for Microbiology)

Note:

- How is genomics different from traditional genetics?

- If you wanted to study how two different cells in the body respond to an infection, what –omics field would you apply?
- What are the biomarkers uncovered in proteomics used for?

Note:

Resolution

Because Kayla's symptoms were persistent and serious enough to interfere with daily activities, Kayla's physician decided to order some laboratory tests. The physician collected samples of Kayla's blood, cerebrospinal fluid (CSF), and synovial fluid (from one of her swollen knees) and requested PCR analysis on all three samples. The PCR tests on the CSF and synovial fluid came back positive for the presence of *Borrelia burgdorferi*, the bacterium that causes Lyme disease.

Kayla's physician immediately prescribed a full course of the antibiotic doxycycline. Fortunately, Kayla recovered fully within a few weeks and did not suffer from the long-term symptoms of post-treatment Lyme disease syndrome (PTLDS), which affects 10–20% of Lyme disease patients. To prevent future infections, Kayla's physician advised her to use insect repellent and wear protective clothing during her outdoor adventures. These measures can limit exposure to Lyme-bearing ticks, which are common in many regions of the United States during the warmer months of the year. Kayla was also advised to make a habit of examining herself for ticks after returning from outdoor activities, as prompt removal of a tick greatly reduces the chances of infection.

Lyme disease is often difficult to diagnose. *B. burgdorferi* is not easily cultured in the laboratory, and the initial symptoms can be very mild and resemble those of many other diseases. But left untreated, the symptoms can become quite severe and debilitating. In addition to two antibody tests, which were inconclusive in Kayla's case, and the PCR test, a Southern blot could be used with *B. burgdorferi*-specific DNA probes to identify DNA from the pathogen. Sequencing of surface protein genes of *Borrelia* species is also being used to identify strains within the species that may be more readily transmitted to humans or cause more severe disease.

Go back to the [previous](#) Clinical Focus box.

Recombinant DNA Technology and Pharmaceutical Production

Genetic engineering has provided a way to create new pharmaceutical products called **recombinant DNA pharmaceuticals**. Such products include antibiotic drugs, vaccines, and hormones used to treat various diseases. [\[link\]](#) lists examples of recombinant DNA products and their uses.

For example, the naturally occurring antibiotic synthesis pathways of various *Streptomyces* spp., long known for their antibiotic production capabilities, can be modified to improve yields or to create new antibiotics through the introduction of genes encoding additional enzymes. More than 200 new antibiotics have been generated through the targeted inactivation of genes and the novel combination of antibiotic synthesis genes in antibiotic-producing *Streptomyces* hosts. [\[footnote\]](#)

Jose-Luis Adrio and Arnold L. Demain. “Recombinant Organisms for Production of Industrial Products.” *Bioengineered Bugs* 1 no. 2 (2010): 116–131.

Genetic engineering is also used to manufacture subunit vaccines, which are safer than other vaccines because they contain only a single antigenic molecule and lack any part of the genome of the pathogen (see [Vaccines](#)). For example, a vaccine for hepatitis B is created by inserting a gene encoding a hepatitis B surface protein into a yeast; the yeast then produces this protein, which the human immune system recognizes as an antigen. The hepatitis B antigen is purified from yeast cultures and administered to patients as a vaccine. Even though the vaccine does not contain the hepatitis B virus, the presence of the antigenic protein stimulates the immune system to produce antibodies that will protect the patient against the virus in the event of exposure. [\[footnote\]](#) [\[footnote\]](#)

U.S. Department of Health and Human Services. “Types of Vaccines.” 2013. http://www.vaccines.gov/more_info/types/#subunit. Accessed May 27, 2016.

The Internet Drug List. *Recombivax*. 2015.

<http://www.rxlist.com/recombivax-drug.htm>. Accessed May 27, 2016.

Genetic engineering has also been important in the production of other therapeutic proteins, such as insulin, interferons, and human growth

hormone, to treat a variety of human medical conditions. For example, at one time, it was possible to treat diabetes only by giving patients pig insulin, which caused allergic reactions due to small differences between the proteins expressed in human and pig insulin. However, since 1978, recombinant DNA technology has been used to produce large-scale quantities of human insulin using *E. coli* in a relatively inexpensive process that yields a more consistently effective pharmaceutical product. Scientists have also genetically engineered *E. coli* capable of producing human growth hormone (HGH), which is used to treat growth disorders in children and certain other disorders in adults. The HGH gene was cloned from a cDNA library and inserted into *E. coli* cells by cloning it into a bacterial vector. Eventually, genetic engineering will be used to produce DNA vaccines and various gene therapies, as well as customized medicines for fighting cancer and other diseases.

Some Genetically Engineered Pharmaceutical Products and Applications

Recombinant DNA Product	Application
Atrial natriuretic peptide	Treatment of heart disease (e.g., congestive heart failure), kidney disease, high blood pressure
DNase	Treatment of viscous lung secretions in cystic fibrosis
Erythropoietin	Treatment of severe anemia with kidney damage
Factor VIII	Treatment of hemophilia

Some Genetically Engineered Pharmaceutical Products and Applications

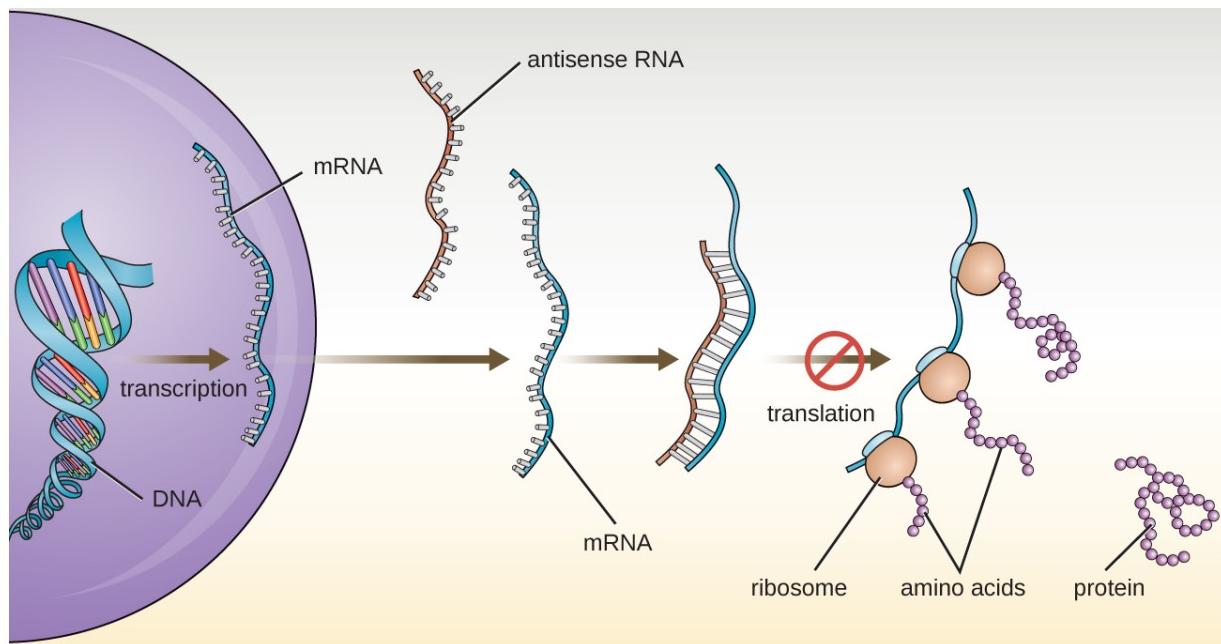
Recombinant DNA Product	Application
Hepatitis B vaccine	Prevention of hepatitis B infection
Human growth hormone	Treatment of growth hormone deficiency, Turner's syndrome, burns
Human insulin	Treatment of diabetes
Interferons	Treatment of multiple sclerosis, various cancers (e.g., melanoma), viral infections (e.g., Hepatitis B and C)
Tetracenomycins	Used as antibiotics
Tissue plasminogen activator	Treatment of pulmonary embolism in ischemic stroke, myocardial infarction

Note:

- What bacterium has been genetically engineered to produce human insulin for the treatment of diabetes?
- Explain how microorganisms can be engineered to produce vaccines.

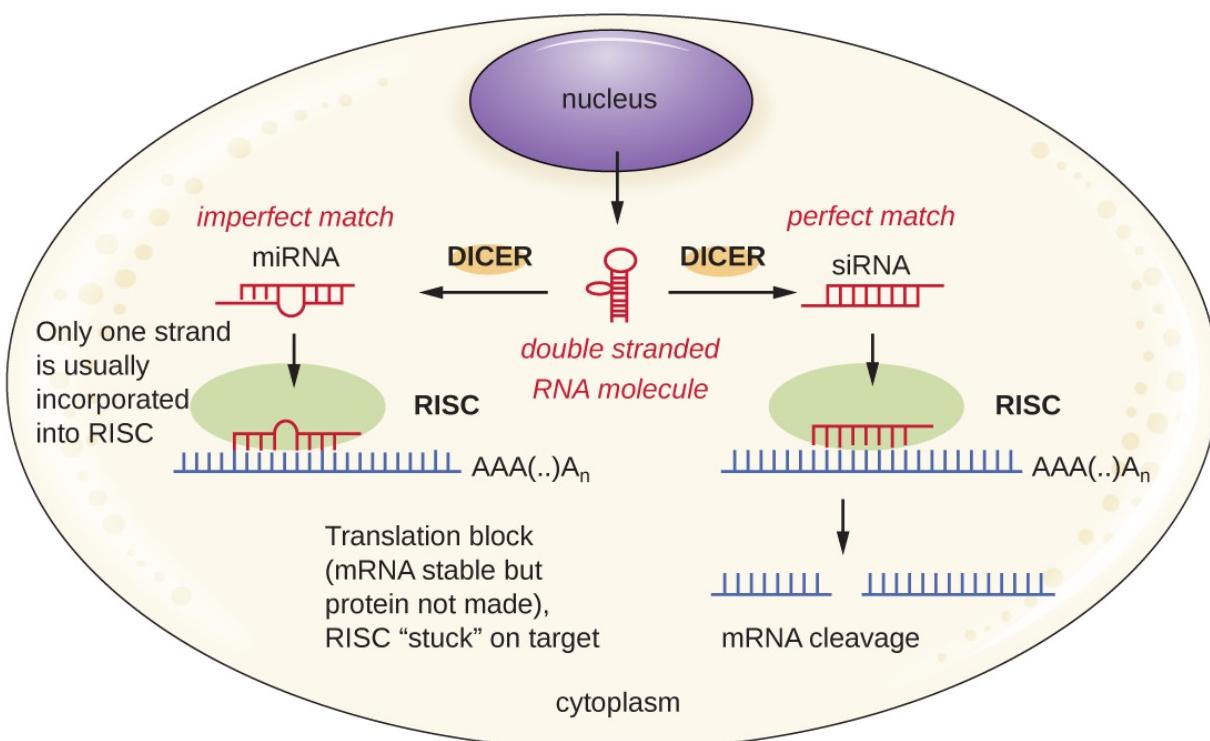
RNA Interference Technology

In [Structure and Function of RNA](#), we described the function of mRNA, rRNA, and tRNA. In addition to these types of RNA, cells also produce several types of small noncoding RNA molecules that are involved in the regulation of gene expression. These include **antisense RNA** molecules, which are complementary to regions of specific mRNA molecules found in both prokaryotes and eukaryotic cells. Non-coding RNA molecules play a major role in **RNA interference (RNAi)**, a natural regulatory mechanism by which mRNA molecules are prevented from guiding the synthesis of proteins. RNA interference of specific genes results from the base pairing of short, single-stranded antisense RNA molecules to regions within complementary mRNA molecules, preventing protein synthesis. Cells use RNA interference to protect themselves from viral invasion, which may introduce double-stranded RNA molecules as part of the viral replication process ([\[link\]](#)).



Cells like the eukaryotic cell shown in this diagram commonly make small antisense RNA molecules with sequences complementary to specific mRNA molecules. When an antisense RNA molecule is bound to an mRNA molecule, the mRNA can no longer be used to direct protein synthesis. (credit: modification of work by Robinson R)

Researchers are currently developing techniques to mimic the natural process of RNA interference as a way to treat viral infections in eukaryotic cells. RNA interference technology involves using small interfering RNAs (siRNAs) or microRNAs (miRNAs) ([\[link\]](#)). siRNAs are completely complementary to the mRNA transcript of a specific gene of interest while miRNAs are mostly complementary. These double-stranded RNAs are bound to DICER, an endonuclease that cleaves the RNA into short molecules (approximately 20 nucleotides long). The RNAs are then bound to RNA-induced silencing complex (RISC), a ribonucleoprotein. The siRNA-RISC complex binds to mRNA and cleaves it. For miRNA, only one of the two strands binds to RISC. The miRNA-RISC complex then binds to mRNA, inhibiting translation. If the miRNA is completely complementary to the target gene, then the mRNA can be cleaved. Taken together, these mechanisms are known as **gene silencing**.



This diagram illustrates the process of using siRNA or miRNA in a

eukaryotic cell to silence genes involved in the pathogenesis of various diseases. (credit: modification of work by National Center for Biotechnology Information)

Key Concepts and Summary

- The science of **genomics** allows researchers to study organisms on a holistic level and has many applications of medical relevance.
- **Transcriptomics** and **proteomics** allow researchers to compare gene expression patterns between different cells and shows great promise in better understanding global responses to various conditions.
- The various –omics technologies complement each other and together provide a more complete picture of an organism's or microbial community's (**metagenomics**) state.
- The analysis required for large data sets produced through genomics, transcriptomics, and **proteomics** has led to the emergence of **bioinformatics**.
- **Reporter genes** encoding easily observable characteristics are commonly used to track gene expression patterns of genes of unknown function.
- The use of recombinant DNA technology has revolutionized the pharmaceutical industry, allowing for the rapid production of high-quality **recombinant DNA pharmaceuticals** used to treat a wide variety of human conditions.
- **RNA interference** technology has great promise as a method of treating viral infections by silencing the expression of specific genes

Multiple Choice

Exercise:

Problem:

The science of studying the entire collection of mRNA molecules produced by cells, allowing scientists to monitor differences in gene expression patterns between cells, is called:

- a. genomics
 - b. transcriptomics
 - c. proteomics
 - d. pharmacogenomics
-

Solution:

B

Exercise:**Problem:**

The science of studying genomic fragments from microbial communities, allowing researchers to study genes from a collection of multiple species, is called:

- a. pharmacogenomics
 - b. transcriptomics
 - c. metagenomics
 - d. proteomics
-

Solution:

C

Exercise:

Problem: The insulin produced by recombinant DNA technology is

- a. a combination of *E. coli* and human insulin.

-
- b. identical to human insulin produced in the pancreas.
 - c. cheaper but less effective than pig insulin for treating diabetes.
 - d. engineered to be more effective than human insulin.
-

Solution:

B

Fill in the Blank

Exercise:

Problem:

The application of genomics to evaluate the effectiveness and safety of drugs on the basis of information from an individual's genomic sequence is called _____.

Solution:

pharmacogenomics or toxicogenomics

Exercise:

Problem:

A gene whose expression can be easily visualized and monitored is called a _____.

Solution:

reporter gene

True/False

Exercise:

Problem:

RNA interference does not influence the sequence of genomic DNA.

Solution:

true

Short answer**Exercise:****Problem:**

If all cellular proteins are encoded by the cell's genes, what information does proteomics provide that genomics cannot?

Critical Thinking**Exercise:****Problem:**

What are some advantages of cloning human genes into bacteria to treat human diseases caused by specific protein deficiencies?

Gene Therapy

LEARNING OBJECTIVES

- Summarize the mechanisms, risks, and potential benefits of gene therapy
- Identify ethical issues involving gene therapy and the regulatory agencies that provide oversight for clinical trials
- Compare somatic-cell and germ-line gene therapy

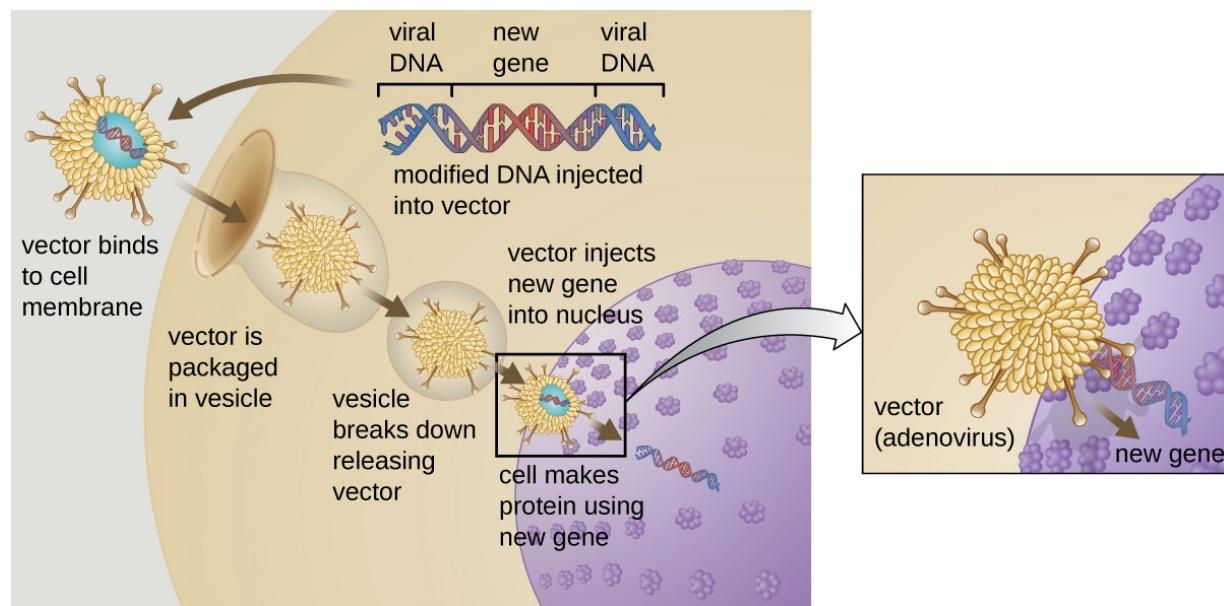
Many types of genetic engineering have yielded clear benefits with few apparent risks. Few would question, for example, the value of our now abundant supply of human insulin produced by genetically engineered bacteria. However, many emerging applications of genetic engineering are much more controversial, often because their potential benefits are pitted against significant risks, real or perceived. This is certainly the case for **gene therapy**, a clinical application of genetic engineering that may one day provide a cure for many diseases but is still largely an experimental approach to treatment.

Mechanisms and Risks of Gene Therapy

Human diseases that result from genetic mutations are often difficult to treat with drugs or other traditional forms of therapy because the signs and symptoms of disease result from abnormalities in a patient's genome. For example, a patient may have a genetic mutation that prevents the expression of a specific protein required for the normal function of a particular cell type. This is the case in patients with Severe Combined Immunodeficiency

(SCID), a genetic disease that impairs the function of certain white blood cells essential to the immune system.

Gene therapy attempts to correct genetic abnormalities by introducing a nonmutated, functional gene into the patient's genome. The nonmutated gene encodes a functional protein that the patient would otherwise be unable to produce. Viral vectors such as adenovirus are sometimes used to introduce the functional gene; part of the viral genome is removed and replaced with the desired gene ([\[link\]](#)). More advanced forms of gene therapy attempt to correct the mutation at the original site in the genome, such as is the case with treatment of SCID.



Gene therapy using an adenovirus vector can be used to treat or cure certain genetic diseases in which a patient has a defective gene. (credit: modification of work by National Institutes of Health)

So far, gene therapies have proven relatively ineffective, with the possible exceptions of treatments for cystic fibrosis and adenosine deaminase deficiency, a type of SCID. Other trials have shown the clear hazards of

attempting genetic manipulation in complex multicellular organisms like humans. In some patients, the use of an adenovirus vector can trigger an unanticipated inflammatory response from the immune system, which may lead to organ failure. Moreover, because viruses can often target multiple cell types, the virus vector may infect cells not targeted for the therapy, damaging these other cells and possibly leading to illnesses such as cancer. Another potential risk is that the modified virus could revert to being infectious and cause disease in the patient. Lastly, there is a risk that the inserted gene could unintentionally inactivate another important gene in the patient's genome, disrupting normal cell cycling and possibly leading to tumor formation and cancer. Because gene therapy involves so many risks, candidates for gene therapy need to be fully informed of these risks before providing informed consent to undergo the therapy.

Note:

Gene Therapy Gone Wrong

The risks of gene therapy were realized in the 1999 case of Jesse Gelsinger, an 18-year-old patient who received gene therapy as part of a clinical trial at the University of Pennsylvania. Jesse received gene therapy for a condition called ornithine transcarbamylase (OTC) deficiency, which leads to ammonia accumulation in the blood due to deficient ammonia processing. Four days after the treatment, Jesse died after a massive immune response to the adenovirus vector.[\[footnote\]](#)

Barbara Sibbald. "Death but One Unintended Consequence of Gene-Therapy Trial." *Canadian Medical Association Journal* 164 no. 11 (2001): 1612–1612.

Until that point, researchers had not really considered an immune response to the vector to be a legitimate risk, but on investigation, it appears that the researchers had some evidence suggesting that this was a possible outcome. Prior to Jesse's treatment, several other human patients had suffered side effects of the treatment, and three monkeys used in a trial had died as a result of inflammation and clotting disorders. Despite this information, it appears that neither Jesse nor his family were made aware of these outcomes when they consented to the therapy. Jesse's death was the first patient death due to a gene therapy treatment and resulted in the

immediate halting of the clinical trial in which he was involved, the subsequent halting of all other gene therapy trials at the University of Pennsylvania, and the investigation of all other gene therapy trials in the United States. As a result, the regulation and oversight of gene therapy overall was reexamined, resulting in new regulatory protocols that are still in place today.

Note:

- Explain how gene therapy works in theory.
- Identify some risks of gene therapy.

Oversight of Gene Therapy

Presently, there is significant oversight of gene therapy clinical trials. At the federal level, three agencies regulate gene therapy in parallel: the Food and Drug Administration (FDA), the Office of Human Research Protection (OHRP), and the Recombinant DNA Advisory Committee (RAC) at the National Institutes of Health (NIH). Along with several local agencies, these federal agencies interact with the institutional review board to ensure that protocols are in place to protect patient safety during clinical trials. Compliance with these protocols is enforced mostly on the local level in cooperation with the federal agencies. Gene therapies are currently under the most extensive federal and local review compared to other types of therapies, which are more typically only under the review of the FDA. Some researchers believe that these extensive regulations actually inhibit progress in gene therapy research. In 2013, the Institute of Medicine (now the National Academy of Medicine) called upon the NIH to relax its review of gene therapy trials in most cases.[\[footnote\]](#) However, ensuring patient safety continues to be of utmost concern.

Kerry Grens. “Report: Ease Gene Therapy Reviews.” *The Scientist*, December 9, 2013. <http://www.the-scientist.com/>?

[articles.view/articleNo/38577/title/Report--Ease-Gene-Therapy-Reviews/](#). Accessed May 27, 2016.

Ethical Concerns

Beyond the health risks of gene therapy, the ability to genetically modify humans poses a number of ethical issues related to the limits of such “therapy.” While current research is focused on gene therapy for genetic diseases, scientists might one day apply these methods to manipulate other genetic traits not perceived as desirable. This raises questions such as:

- Which genetic traits are worthy of being “corrected”?
- Should gene therapy be used for cosmetic reasons or to enhance human abilities?
- Should genetic manipulation be used to impart desirable traits to the unborn?
- Is everyone entitled to gene therapy, or could the cost of gene therapy create new forms of social inequality?
- Who should be responsible for regulating and policing inappropriate use of gene therapies?

The ability to alter reproductive cells using gene therapy could also generate new ethical dilemmas. To date, the various types of gene therapies have been targeted to somatic cells, the non-reproductive cells within the body. Because somatic cell traits are not inherited, any genetic changes accomplished by somatic-cell gene therapy would not be passed on to offspring. However, should scientists successfully introduce new genes to germ cells (eggs or sperm), the resulting traits could be passed on to offspring. This approach, called germ-line gene therapy, could potentially be used to combat heritable diseases, but it could also lead to unintended consequences for future generations. Moreover, there is the question of informed consent, because those impacted by germ-line gene therapy are unborn and therefore unable to choose whether they receive the therapy. For these reasons, the U.S. government does not currently fund research projects investigating germ-line gene therapies in humans.

Note:

Risky Gene Therapies

While there are currently no gene therapies on the market in the United States, many are in the pipeline and it is likely that some will eventually be approved. With recent advances in gene therapies targeting p53, a gene whose somatic cell mutations have been implicated in over 50% of human cancers,[\[footnote\]](#) cancer treatments through gene therapies could become much more widespread once they reach the commercial market.

Zhen Wang and Yi Sun. “Targeting p53 for Novel Anticancer Therapy.” *Translational Oncology* 3, no. 1 (2010): 1–12.

Bringing any new therapy to market poses ethical questions that pit the expected benefits against the risks. How quickly should new therapies be brought to the market? How can we ensure that new therapies have been sufficiently tested for safety and effectiveness before they are marketed to the public? The process by which new therapies are developed and approved complicates such questions, as those involved in the approval process are often under significant pressure to get a new therapy approved even in the face of significant risks.

To receive FDA approval for a new therapy, researchers must collect significant laboratory data from animal trials and submit an Investigational New Drug (IND) application to the FDA’s Center for Drug Evaluation and Research (CDER). Following a 30-day waiting period during which the FDA reviews the IND, clinical trials involving human subjects may begin. If the FDA perceives a problem prior to or during the clinical trial, the FDA can order a “clinical hold” until any problems are addressed. During clinical trials, researchers collect and analyze data on the therapy’s effectiveness and safety, including any side effects observed. Once the therapy meets FDA standards for effectiveness and safety, the developers can submit a New Drug Application (NDA) that details how the therapy will be manufactured, packaged, monitored, and administered.

Because new gene therapies are frequently the result of many years (even decades) of laboratory and clinical research, they require a significant financial investment. By the time a therapy has reached the clinical trials stage, the financial stakes are high for pharmaceutical companies and their shareholders. This creates potential conflicts of interest that can sometimes affect the objective judgment of researchers, their funders, and even trial participants. The Jesse Gelsinger case (see [Case in Point: Gene Therapy](#))

[Gone Wrong](#)) is a classic example. Faced with a life-threatening disease and no reasonable treatments available, it is easy to see why a patient might be eager to participate in a clinical trial no matter the risks. It is also easy to see how a researcher might view the short-term risks for a small group of study participants as a small price to pay for the potential benefits of a game-changing new treatment.

Gelsinger's death led to increased scrutiny of gene therapy, and subsequent negative outcomes of gene therapy have resulted in the temporary halting of clinical trials pending further investigation. For example, when children in France treated with gene therapy for SCID began to develop leukemia several years after treatment, the FDA temporarily stopped clinical trials of similar types of gene therapy occurring in the United States.[\[footnote\]](#) Cases like these highlight the need for researchers and health professionals not only to value human well-being and patients' rights over profitability, but also to maintain scientific objectivity when evaluating the risks and benefits of new therapies.

Erika Check. "Gene Therapy: A Tragic Setback." *Nature* 420 no. 6912 (2002): 116–118.

Note:

- Why is gene therapy research so tightly regulated?
- What is the main ethical concern associated with germ-line gene therapy?

Key Concepts and Summary

- While gene therapy shows great promise for the treatment of genetic diseases, there are also significant risks involved.
- There is considerable federal and local regulation of the development of gene therapies by pharmaceutical companies for use in humans.
- Before gene therapy use can increase dramatically, there are many ethical issues that need to be addressed by the medical and research

communities, politicians, and society at large.

Multiple Choice

Exercise:

Problem:

At what point can the FDA halt the development or use of gene therapy?

- a. on submission of an IND application
- b. during clinical trials
- c. after manufacturing and marketing of the approved therapy
- d. all of the answers are correct

Solution:

D

Fill in the Blank

Exercise:

Problem:

_____ is a common viral vector used in gene therapy for introducing a new gene into a specifically targeted cell type.

Solution:

Adenovirus

Short Answer

Exercise:

Problem:

Briefly describe the risks associated with somatic cell gene therapy.

Critical Thinking

Exercise:

Problem:

Compare the ethical issues involved in the use of somatic cell gene therapy and germ-line gene therapy.

Introduction class="introduction"

Malaria is a disease caused by a eukaryotic parasite transmitted to humans by mosquitos.

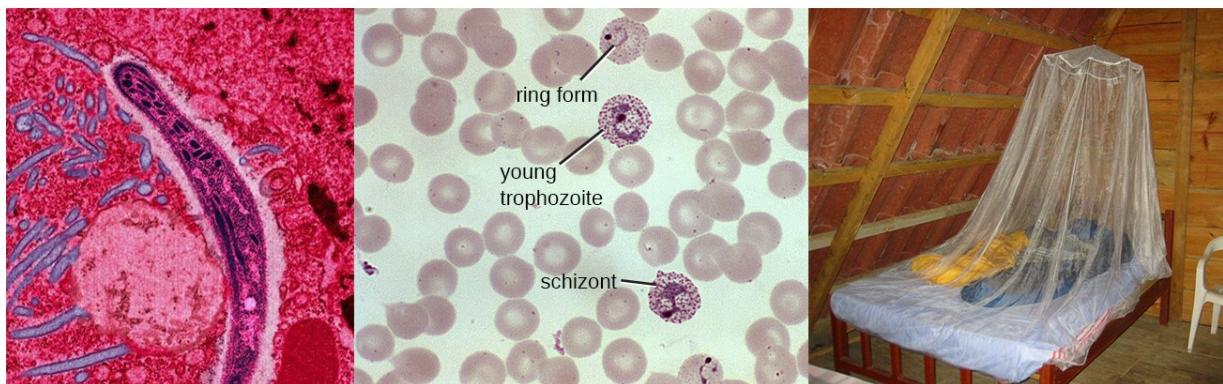
Micrographs (left and center) show a sporozoite life stage, trophozoites

, and a schizont in a blood smear.

On the right is depicted a primary defense against mosquito-borne illnesses like malaria—mosquito netting.

(credit left: modification of work by Ute Frevert; credit

middle:
modification
of work by
Centers for
Disease
Control and
Prevention;
credit right:
modification
of work by
Tjeerd
Wiersma)



Although bacteria and viruses account for a large number of the infectious diseases that afflict humans, many serious illnesses are caused by eukaryotic organisms. One example is malaria, which is caused by *Plasmodium*, a eukaryotic organism transmitted through mosquito bites. Malaria is a major cause of morbidity (illness) and mortality (death) that threatens 3.4 billion people worldwide.[\[footnote\]](#) In severe cases, organ failure and blood or metabolic abnormalities contribute to medical emergencies and sometimes death. Even after initial recovery, relapses may occur years later. In countries where malaria is endemic, the disease represents a major public health challenge that can place a tremendous strain on developing economies.

Centers for Disease Control and Prevention. “Impact of Malaria.” September 22, 2015.

http://www.cdc.gov/malaria/malaria_worldwide/impact.html. Accessed January 18, 2016.

Worldwide, major efforts are underway to reduce malaria infections. Efforts include the distribution of insecticide-treated bed nets and the spraying of pesticides. Researchers are also making progress in their efforts to develop effective vaccines.[\[footnote\]](#) The President's Malaria Initiative, started in 2005, supports prevention and treatment. The Bill and Melinda Gates Foundation has a large initiative to eliminate malaria. Despite these efforts, malaria continues to cause long-term morbidity (such as intellectual disabilities in children) and mortality (especially in children younger than 5 years), so we still have far to go.

RTS, S Clinical Trials Partnership. "Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial." *The Lancet* 23 April 2015. DOI: [http://dx.doi.org/10.1016/S0140-6736\(15\)60721-8](http://dx.doi.org/10.1016/S0140-6736(15)60721-8).

Protozoa

LEARNING OBJECTIVES

- Summarize the general characteristics of unicellular eukaryotic parasites
- Describe the general life cycles and modes of reproduction in unicellular eukaryotic parasites
- Identify challenges associated with classifying unicellular eukaryotes
- Explain the taxonomic scheme used for unicellular eukaryotes
- Give examples of infections caused by unicellular eukaryotes

Note:

Part 1

Upon arriving home from school, 7-year-old Sarah complains that a large spot on her arm will not stop itching. She keeps scratching at it, drawing the attention of her parents. Looking more closely, they see that it is a red circular spot with a raised red edge ([\[link\]](#)). The next day, Sarah's parents take her to their doctor, who examines the spot using a Wood's lamp. A Wood's lamp produces ultraviolet light that causes the spot on Sarah's arm to fluoresce, which confirms what the doctor already suspected: Sarah has a case of ringworm.

Sarah's mother is mortified to hear that her daughter has a "worm." How could this happen?

- What are some likely ways that Sarah might have contracted ringworm?

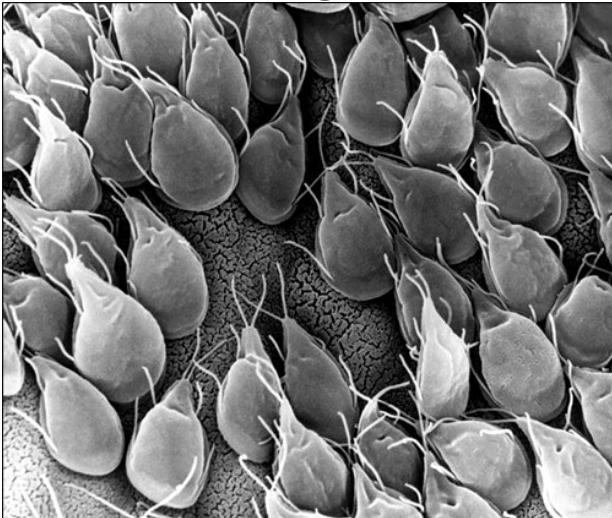


Ringworm presents as a raised, red ring on the skin.
(credit: Centers for Disease Control and Prevention)

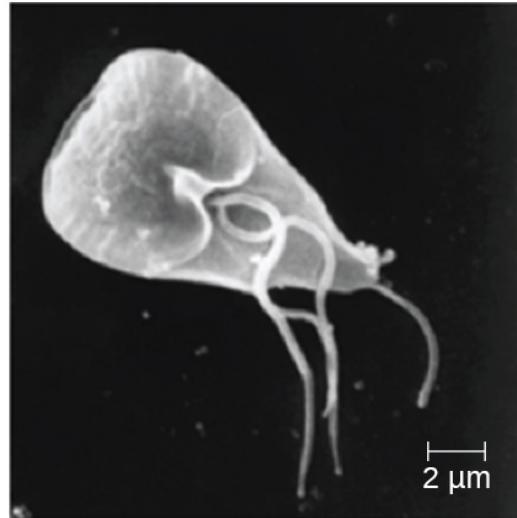
Jump to the [next](#) Clinical Focus box.

Eukaryotic microbes are an extraordinarily diverse group, including species with a wide range of life cycles, morphological specializations, and nutritional needs. Although more diseases are caused by viruses and bacteria than by microscopic eukaryotes, these eukaryotes are responsible for some diseases of great public health importance. For example, the protozoal disease malaria was responsible for 584,000 deaths worldwide (primarily children in Africa) in 2013, according to the World Health Organization (WHO). The protist parasite *Giardia* causes a diarrheal illness (giardiasis) that is easily transmitted through contaminated water supplies. In the United States, *Giardia* is the most common human intestinal parasite ([\[link\]](#)). Although it may seem surprising, parasitic worms are included within the study of microbiology because identification depends on observation of microscopic adult worms or eggs. Even in developed

countries, these worms are important parasites of humans and of domestic animals. There are fewer fungal pathogens, but these are important causes of illness, as well. On the other hand, fungi have been important in producing antimicrobial substances such as penicillin. In this chapter, we will examine characteristics of protists, worms, and fungi while considering their roles in causing disease.



(a)



(b)

(a) A scanning electron micrograph shows many *Giardia* parasites in the trophozoite, or feeding stage, in a gerbil intestine. (b) An individual trophozoite of *G. lamblia*, visualized here in a scanning electron micrograph. This waterborne protist causes severe diarrhea when ingested. (credit a, b: modification of work by Centers for Disease Control and Prevention)

Characteristics of Protists

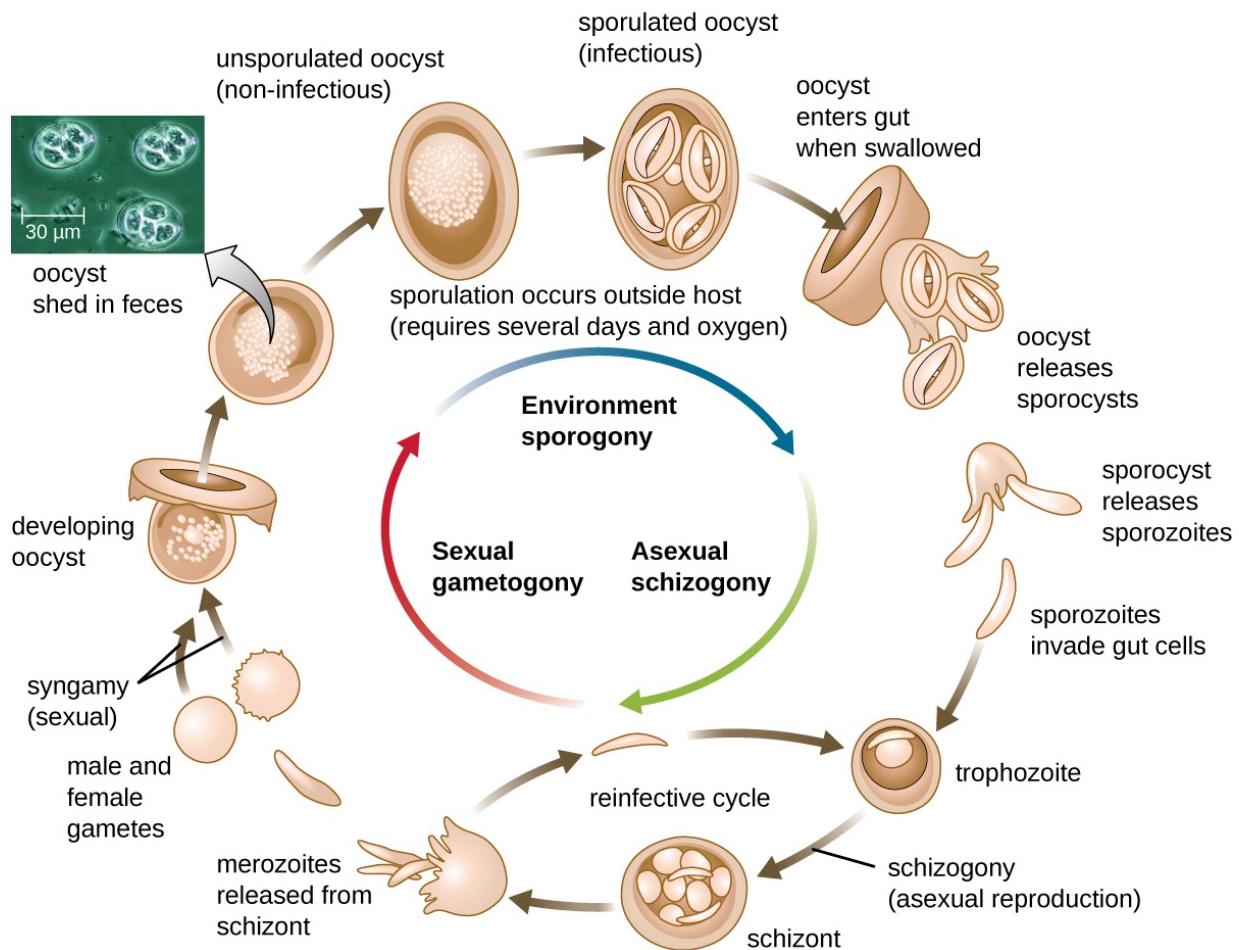
The word *protist* is a historical term that is now used informally to refer to a diverse group of microscopic eukaryotic organisms. It is not considered a formal taxonomic term because the organisms it describes do not have a shared evolutionary origin. Historically, the protists were informally grouped into the “animal-like” protozoans, the “plant-like” algae, and the “fungus-like” protists such as water molds. These three groups of protists

differ greatly in terms of their basic characteristics. For example, algae are photosynthetic organisms that can be unicellular or multicellular. Protozoa, on the other hand, are nonphotosynthetic, motile organisms that are always unicellular. Other informal terms may also be used to describe various groups of protists. For example, microorganisms that drift or float in water, moved by currents, are referred to as **plankton**. Types of plankton include **zooplankton**, which are motile and nonphotosynthetic, and **phytoplankton**, which are photosynthetic.

Protozoans inhabit a wide variety of habitats, both aquatic and terrestrial. Many are free-living, while others are parasitic, carrying out a life cycle within a host or hosts and potentially causing illness. There are also beneficial symbionts that provide metabolic services to their hosts. During the feeding and growth part of their life cycle, they are called **trophozoites**; these feed on small particulate food sources such as bacteria. While some types of protozoa exist exclusively in the trophozoite form, others can develop from trophozoite to an encapsulated cyst stage when environmental conditions are too harsh for the trophozoite. A **cyst** is a cell with a protective wall, and the process by which a trophozoite becomes a cyst is called **encystment**. When conditions become more favorable, these cysts are triggered by environmental cues to become active again through **excystment**.

One protozoan genus capable of encystment is *Eimeria*, which includes some human and animal pathogens. [\[link\]](#) illustrates the life cycle of *Eimeria*.

Eimeria Life Cycle



In the sexual/sexual life cycle of *Eimeria*, oocysts (inset) are shed in feces and may cause disease when ingested by a new host. (credit “life cycle,” “micrograph”: modification of work by USDA)

Protozoans have a variety of reproductive mechanisms. Some protozoans reproduce asexually and others reproduce sexually; still others are capable of both sexual and asexual reproduction. In protozoans, asexual reproduction occurs by binary fission, budding, or schizogony. In **schizogony**, the nucleus of a cell divides multiple times before the cell divides into many smaller cells. The products of schizogony are called merozoites and they are stored in structures known as schizonts. Protozoans may also reproduce sexually, which increases genetic diversity and can lead

to complex life cycles. Protozoans can produce haploid gametes that fuse through **syngamy**. However, they can also exchange genetic material by joining to exchange DNA in a process called conjugation. This is a different process than the conjugation that occurs in bacteria. The term protist conjugation refers to a true form of eukaryotic sexual reproduction between two cells of different mating types. It is found in **ciliates**, a group of protozoans, and is described later in this subsection.

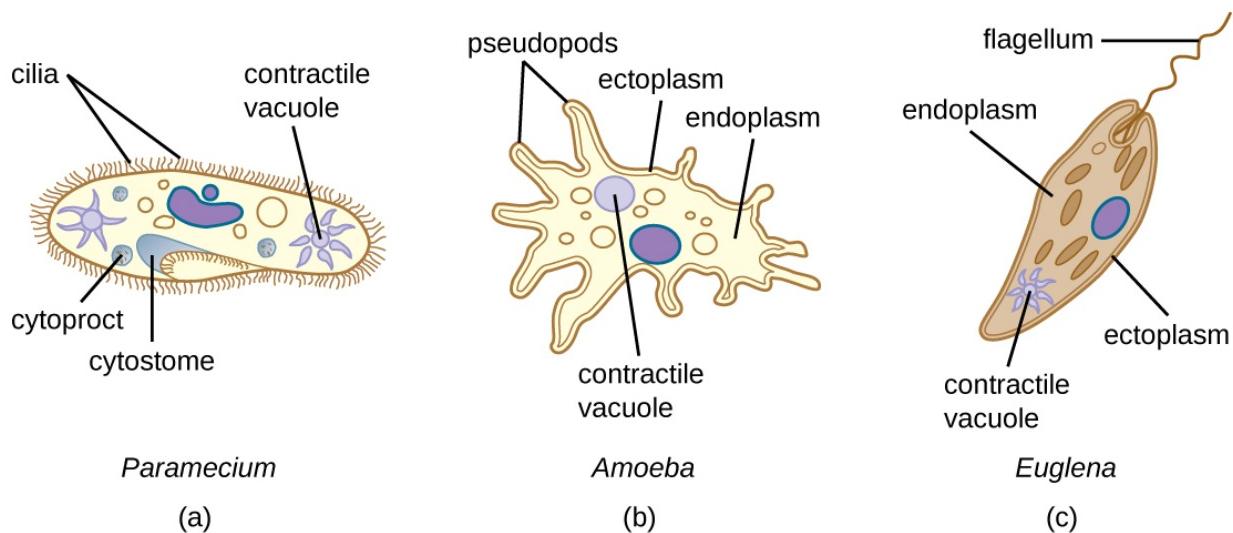
All protozoans have a plasma membrane, or **plasmalemma**, and some have bands of protein just inside the membrane that add rigidity, forming a structure called the **pellicle**. Some protists, including protozoans, have distinct layers of cytoplasm under the membrane. In these protists, the outer gel layer (with microfilaments of actin) is called the **ectoplasm**. Inside this layer is a sol (fluid) region of cytoplasm called the **endoplasm**. These structures contribute to complex cell shapes in some protozoans, whereas others (such as amoebas) have more flexible shapes ([\[link\]](#)).

Different groups of protozoans have specialized feeding structures. They may have a specialized structure for taking in food through phagocytosis, called a **cytostome**, and a specialized structure for the exocytosis of wastes called a **cytoproct**. Oral grooves leading to cytostomes are lined with hair-like cilia to sweep in food particles. Protozoans are heterotrophic. Protozoans that are **holozoic** ingest whole food particles through phagocytosis. Forms that are **saprozoic** ingest small, soluble food molecules.

Many protists have whip-like flagella or hair-like cilia made of microtubules that can be used for locomotion ([\[link\]](#)). Other protists use cytoplasmic extensions known as pseudopodia (“false feet”) to attach the cell to a surface; they then allow cytoplasm to flow into the extension, thus moving themselves forward.

Protozoans have a variety of unique organelles and sometimes lack organelles found in other cells. Some have **contractile vacuoles**, organelles that can be used to move water out of the cell for osmotic regulation (salt and water balance) ([\[link\]](#)). Mitochondria may be absent in parasites or altered to kinetoplastids (modified mitochondria) or hydrogenosomes (see

[Unique Characteristics of Prokaryotic Cells](#) for more discussion of these structures).



(a) *Paramecium* spp. have hair-like appendages called cilia for locomotion. (b) *Amoeba* spp. use lobe-like pseudopodia to anchor the cell to a solid surface and pull forward. (c) *Euglena* spp. use a whip-like structure called a flagellum to propel the cell.

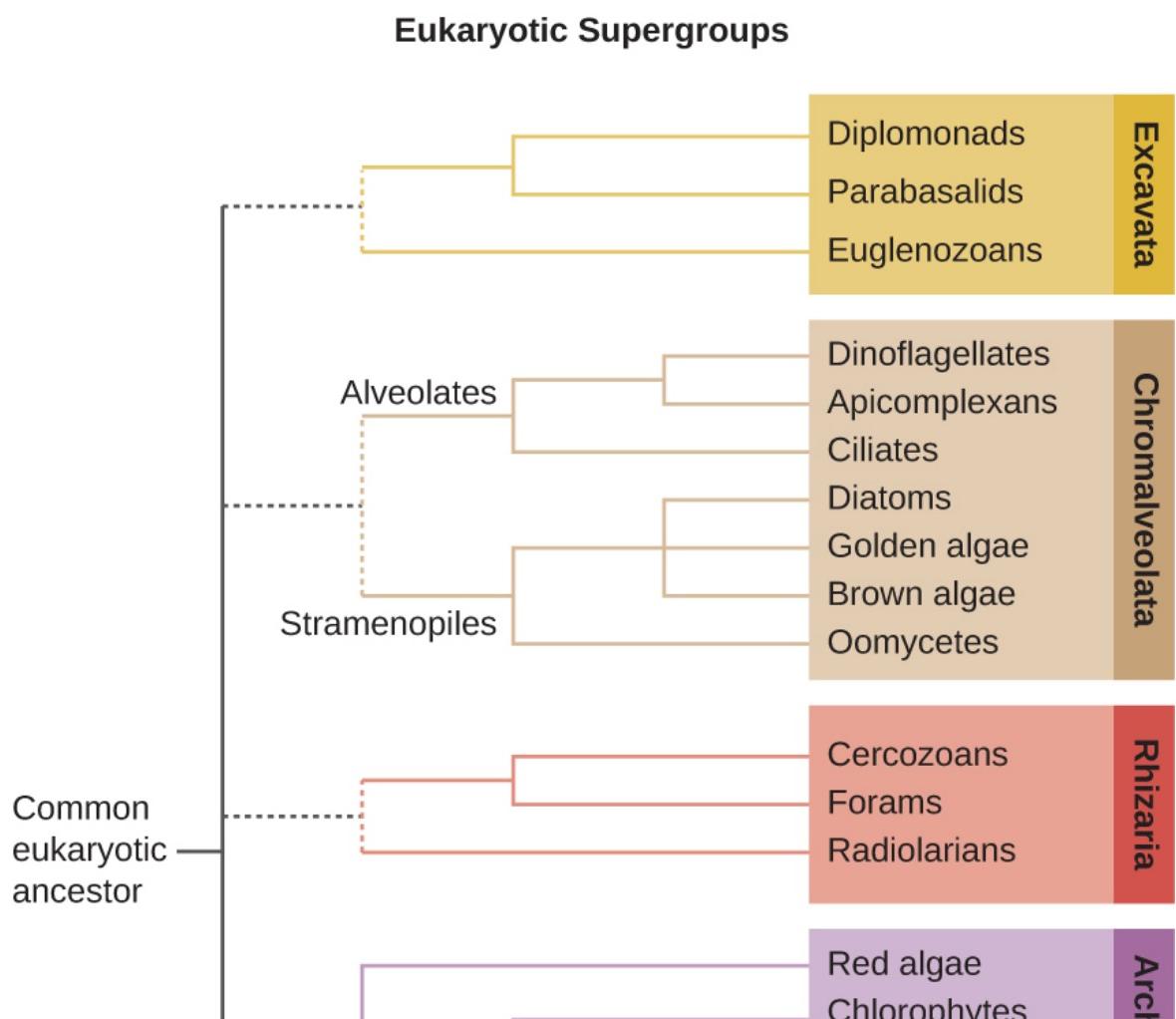
Note:

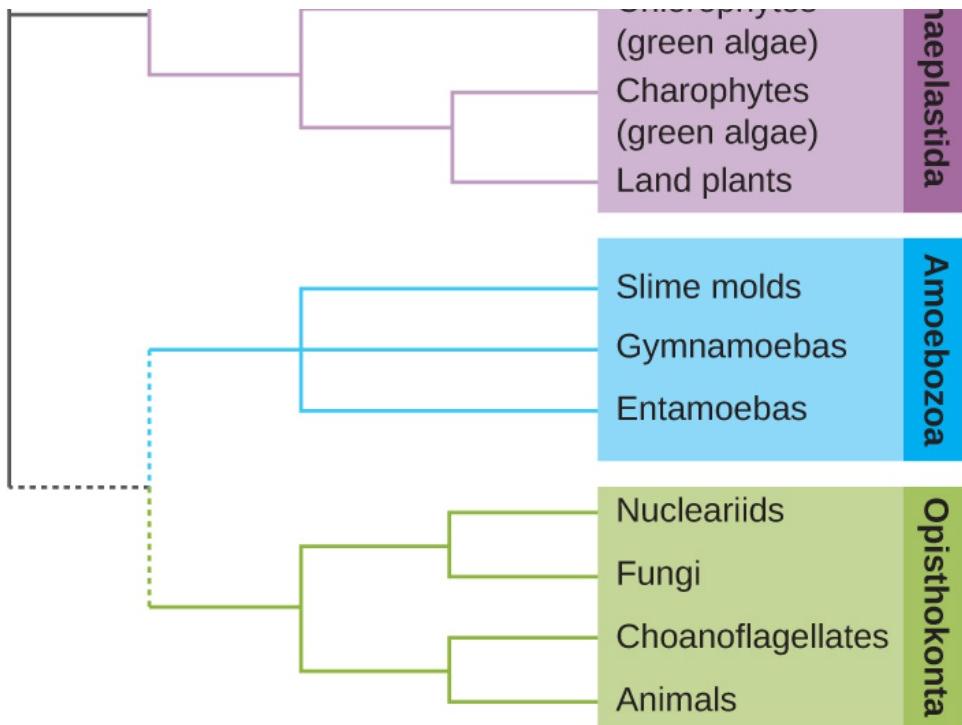
- What is the sequence of events in reproduction by schizogony and what are the cells produced called?

Taxonomy of Protists

The protists are a **polyphyletic** group, meaning they lack a shared evolutionary origin. Since the current taxonomy is based on evolutionary

history (as determined by biochemistry, morphology, and genetics), protists are scattered across many different taxonomic groups within the domain Eukarya. Eukarya is currently divided into six supergroups that are further divided into subgroups, as illustrated in ([\[link\]](#)). In this section, we will primarily be concerned with the supergroups Amoebozoa, Excavata, and Chromalveolata; these supergroups include many protozoans of clinical significance. The supergroups Opisthokonta and Rhizaria also include some protozoans, but few of clinical significance. In addition to protozoans, Opisthokonta also includes animals and fungi, some of which we will discuss in [Parasitic Helminths](#) and [Fungi](#). Some examples of the Archaeplastida will be discussed in [Algae](#). [\[link\]](#) and [\[link\]](#) summarize the characteristics of each supergroup and subgroup and list representatives of each.





This tree shows a proposed classification of the domain Eukarya based on evolutionary relationships. Currently, the domain Eukarya is divided into six supergroups. Within each supergroup are multiple kingdoms. Dotted lines indicate suggested evolutionary relationships that remain under debate.

The Eukaryote Supergroups and Some Examples				
Supergroup	Subgroups	Distinguishing Features	Examples	Clinical Notes
Excavata	Fornicata	Form cysts Pair of equal nuclei No mitochondria Often parasitic Four free flagella	<i>Giardia lamblia</i>	Giardiasis
	Parabasalids	No mitochondria Four free flagella One attached flagellum No cysts Parasitic or symbiotic Basal bodies Kinetoplastids	<i>Trichomonas</i>	Trichomoniasis
	Euglenozoans	Photosynthetic or heterotrophic Flagella	<i>Euglena</i>	N/a
			<i>Trypanosoma</i>	African sleeping sickness, Chagas disease
			<i>Leishmania</i>	Leishmaniasis
Chromalveolata	Dinoflagellates	Cellulose theca Two dissimilar flagella	<i>Gonyaulax</i>	Red tides
			<i>Alexandrium</i>	Paralytic shellfish poisoning
			<i>Pfiesteria</i>	Harmful algal blooms
	Apicomplexans	Intracellular parasite Apical organelles	<i>Plasmodium</i>	Malaria
			<i>Cryptosporidium</i>	Cryptosporidiosis
			<i>Theileria (Babesia)</i>	Babesiosis
			<i>Toxoplasma</i>	Toxoplasmosis
	Ciliates	Cilia	<i>Balantidium</i>	Balantidiasis
			<i>Paramecium</i>	N/a
			<i>Stentor</i>	N/a
	Öomycetes/ peronosporomycetes	“Water molds” Generally diploid Cellulose cell walls	<i>Phytophthora</i>	Diseases in crops

The Eukaryote Supergroups and Some Examples (continued)

Supergroup	Subgroups	Distinguishing Features	Examples	Clinical Notes
Rhizaria	Foraminifera	Amoeboid Threadlike pseudopodia Calcium carbonate shells	<i>Astrolonche</i>	N/a
	Radiolaria	Amoeboid Threadlike pseudopodia Silica shells	<i>Actinomma</i>	N/a
	Cercozoa	Amoeboid Threadlike pseudopodia Complex shells Parasitic forms	<i>Spongospora subterranea</i> <i>Plasmodiophora brassicae</i>	Powdery scab (potato disease) Cabbage clubroot
Archaeplastida	Red algae	Chlorophyll a Phycoerythrin Phycocyanin Floridean starch Agar in cell walls	<i>Gelidium</i> <i>Gracilaria</i>	Source of agar
	Chlorophytes	Chlorophyll a Chlorophyll b Cellulose cell walls Starch storage	<i>Acetabularia</i>	N/a
			<i>Ulva</i>	N/a
	Slime molds	Plasmodial and cellular forms	<i>Dictyostelium</i>	N/a
Amoebozoa	Entamoebas	Trophozoites Form cysts	<i>Entamoeba</i>	Amoebiasis
			<i>Naegleria</i>	Primary amoebic meningoencephalitis
			<i>Acanthamoeba</i>	Keratitis, granulomatous amoebic encephalitis
			Zygomycetes Ascomycetes Basidiomycetes Microsporidia	Zygomycosis Candidiasis Cryptococcosis Microsporidiosis
	Animals	Multicellular heterotrophs No cell walls	Nematoda Trematoda Cestoda	Trichinosis; hookworm and pinworm infections Schistosomiasis Tapeworm infections

Note:

- Which supergroups contain the clinically significant protists?

Amoebozoa

The supergroup Amoebozoa includes protozoans that use amoeboid movement. Actin microfilaments produce pseudopodia, into which the remainder of the protoplasm flows, thereby moving the organism. The genus *Entamoeba* includes commensal or parasitic species, including the medically important *E. histolytica*, which is transmitted by cysts in feces and is the primary cause of amoebic dysentery. The notorious “brain-eating amoeba,” *Naegleria fowleri*, is also classified within the Amoebozoa. This deadly parasite is found in warm, fresh water and causes primary amoebic meningoencephalitis (PAM). Another member of this group is *Acanthamoeba*, which can cause keratitis (corneal inflammation) and blindness.

The Eumycetozoa are an unusual group of organisms called slime molds, which have previously been classified as animals, fungi, and plants ([\[link\]](#)). Slime molds can be divided into two types: cellular slime molds and plasmodial slime molds. The cellular slime molds exist as individual amoeboid cells that periodically aggregate into a mobile slug. The aggregate then forms a fruiting body that produces haploid spores. Plasmodial slime molds exist as large, multinucleate amoeboid cells that form reproductive stalks to produce spores that divide into gametes. One cellular slime mold, *Dictyostelium discoideum*, has been an important study organism for understanding cell differentiation, because it has both single-celled and multicelled life stages, with the cells showing some degree of differentiation in the multicelled form. [\[link\]](#) and [\[link\]](#) illustrate the life cycles of cellular and plasmodial slime molds, respectively.



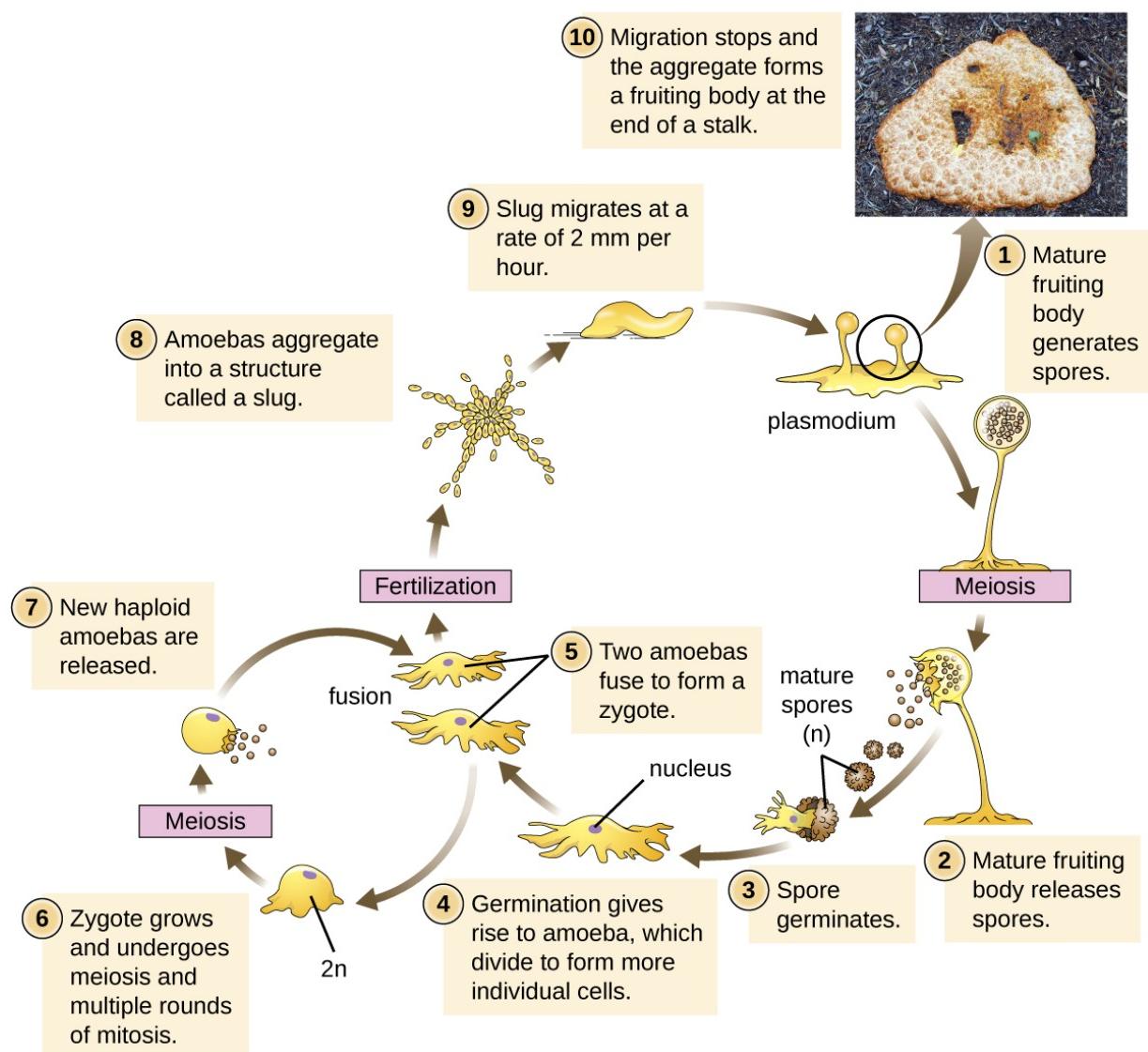
(a)



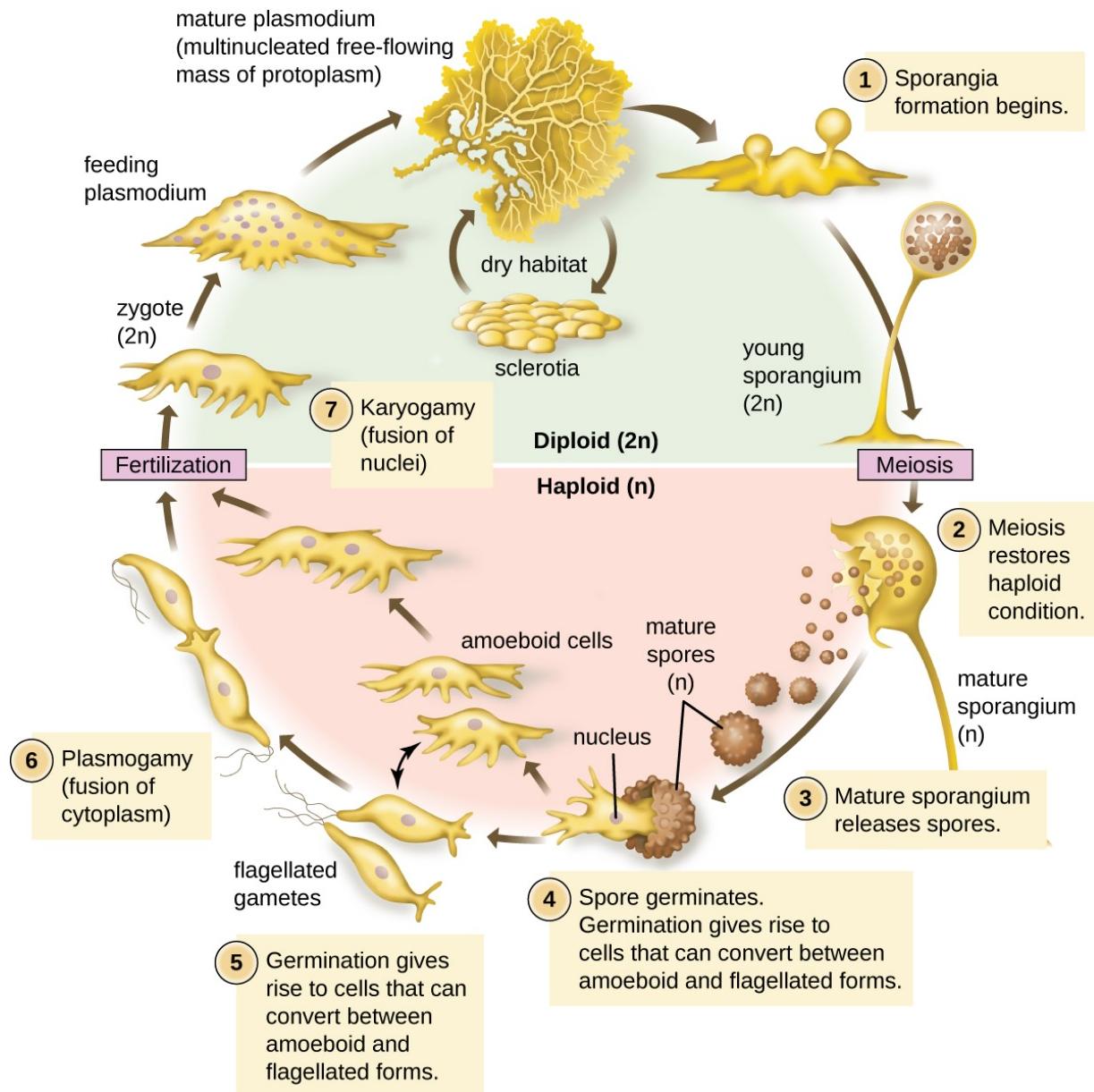
(b)

(a) The cellular slime mold *Dictyostelium discoideum* can be grown on agar in a Petri dish. In this image, individual amoeboid cells (visible as small spheres) are streaming together to form an aggregation that is beginning to rise in the upper right corner of the image. The primitively multicellular aggregation consists of individual cells that each have their own nucleus. (b) *Fuligo septica* is a plasmodial slime mold. This brightly colored organism consists of a large cell with many nuclei.

Haploid and Asexual Reproduction



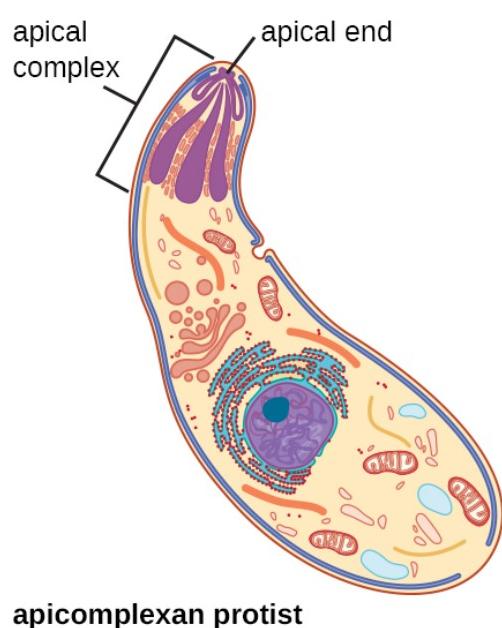
The life cycle of the cellular slime mold *Dictyostelium discoideum* primarily involves individual amoebas but includes the formation of a multinucleate plasmodium formed from a uninucleate zygote (the result of the fusion of two individual amoeboid cells). The plasmodium is able to move and forms a fruiting body that generates haploid spores. (credit “photo”: modification of work by “thatredhead4”/Flickr)



Plasmodial slime molds exist as large multinucleate amoeboid cells that form reproductive stalks to produce spores that divide into gametes.

Chromalveolata

The supergroup Chromalveolata is united by similar origins of its members' plastids and includes the apicomplexans, ciliates, diatoms, and dinoflagellates, among other groups (we will cover the diatoms and dinoflagellates in [Algae](#)). The apicomplexans are intra- or extracellular parasites that have an apical complex at one end of the cell. The apical complex is a concentration of organelles, vacuoles, and microtubules that allows the parasite to enter host cells ([\[link\]](#)). Apicomplexans have complex life cycles that include an infective sporozoite that undergoes schizogony to make many merozoites (see the example in [\[link\]](#)). Many are capable of infecting a variety of animal cells, from insects to livestock to humans, and their life cycles often depend on transmission between multiple hosts. The genus *Plasmodium* is an example of this group.



apicomplexan protist

(a)



(b)

(a) Apicomplexans are parasitic protists. They have a characteristic apical complex that enables them to infect host cells. (b) A colorized electron microscope image of a *Plasmodium* sporozoite. (credit b: modification of work by Ute Frevert)

Other apicomplexans are also medically important. *Cryptosporidium parvum* causes intestinal symptoms and can cause epidemic diarrhea when the cysts contaminate drinking water. *Theileria (Babesia) microti*, transmitted by the tick *Ixodes scapularis*, causes recurring fever that can be fatal and is becoming a common transfusion-transmitted pathogen in the United States (*Theileria* and *Babesia* are closely related genera and there is some debate about the best classification). Finally, *Toxoplasma gondii* causes toxoplasmosis and can be transmitted from cat feces, unwashed fruit and vegetables, or from undercooked meat. Because toxoplasmosis can be associated with serious birth defects, pregnant women need to be aware of this risk and use caution if they are exposed to the feces of potentially infected cats. A national survey found the frequency of individuals with antibodies for toxoplasmosis (and thus who presumably have a current latent infection) in the United States to be 11%. Rates are much higher in other countries, including some developed countries. [footnote] There is also evidence and a good deal of theorizing that the parasite may be responsible for altering infected humans' behavior and personality traits. [footnote]

J. Flegr et al. "Toxoplasmosis—A Global Threat. Correlation of Latent Toxoplasmosis With Specific Disease Burden in a Set of 88 Countries." *PloS ONE* 9 no. 3 (2014):e90203.

J. Flegr. "Effects of Toxoplasma on Human Behavior." *Schizophrenia Bull* 33, no. 3 (2007):757–760.

The ciliates (Ciliaphora), also within the Chromalveolata, are a large, very diverse group characterized by the presence of cilia on their cell surface. Although the cilia may be used for locomotion, they are often used for feeding, as well, and some forms are nonmotile. *Balantidium coli* ([link]) is the only parasitic ciliate that affects humans by causing intestinal illness, although it rarely causes serious medical issues except in the immunocompromised (those having a weakened immune system). Perhaps the most familiar ciliate is *Paramecium*, a motile organism with a clearly visible cytostome and cytoproct that is often studied in biology laboratories ([link]). Another ciliate, *Stentor*, is sessile and uses its cilia for feeding ([link]). Generally, these organisms have a **micronucleus** that is diploid, somatic, and used for sexual reproduction by conjugation. They also have a **macronucleus** that is derived from the micronucleus; the macronucleus

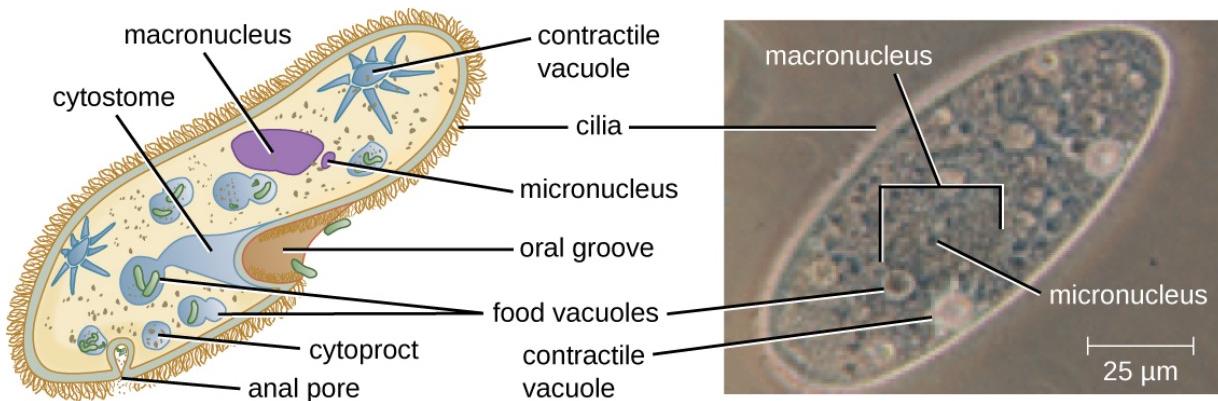
becomes polyplloid (multiple sets of duplicate chromosomes), and has a reduced set of metabolic genes.

Ciliates are able to reproduce through conjugation, in which two cells attach to each other. In each cell, the diploid micronuclei undergo meiosis, producing eight haploid nuclei each. Then, all but one of the haploid micronuclei and the macronucleus disintegrate; the remaining (haploid) micronucleus undergoes mitosis. The two cells then exchange one micronucleus each, which fuses with the remaining micronucleus present to form a new, genetically different, diploid micronucleus. The diploid micronucleus undergoes two mitotic divisions, so each cell has four micronuclei, and two of the four combine to form a new macronucleus. The chromosomes in the macronucleus then replicate repeatedly, the macronucleus reaches its polyplloid state, and the two cells separate. The two cells are now genetically different from each other and from their previous versions.

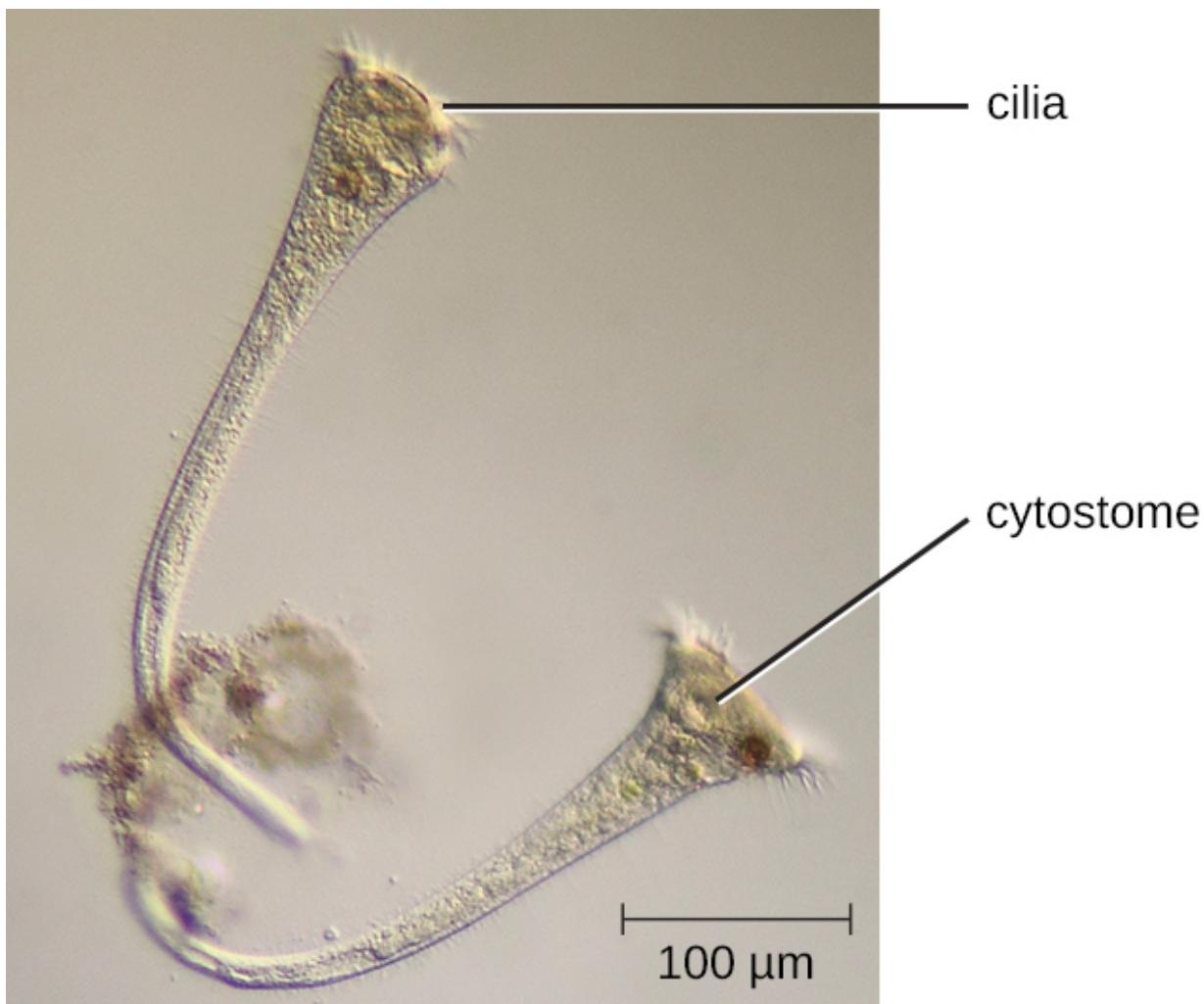


This specimen of the ciliate *Balantidium coli* is a

trophozoite form isolated from the gut of a primate. *B. coli* is the only ciliate capable of parasitizing humans.
(credit: modification of work by Kouassi RYW, McGraw SW, Yao PK, Abou-Bacar A, Brunet J, Pesson B, Bonfoh B, N'goran EK & Candolfi E)



Paramecium has a primitive mouth (called an oral groove) to ingest food, and an anal pore to excrete it. Contractile vacuoles allow the organism to excrete excess water. Cilia enable the organism to move.



This differential interference contrast micrograph (magnification: $\times 65$) of *Stentor roeselie* shows cilia present on the margins of the structure surrounding the cytostome; the cilia move food particles. (credit: modification of work by "picturepest"/Flickr)

Öomycetes have similarities to fungi and were once classified with them. They are also called water molds. However, they differ from fungi in several important ways. Öomycetes have cell walls of cellulose (unlike the chitinous cell walls of fungi) and they are generally diploid, whereas the dominant life forms of fungi are typically haploid. *Phytophthora*, the plant pathogen found in the soil that caused the Irish potato famine, is classified within this group ([\[link\]](#)).



A saprobic oomycete, or water mold, engulfs a dead insect. (credit: modification of work by Thomas Bresson)

Note:



Explore the procedures for detecting the presence of an apicomplexan in a public water supply, at [this](#) website.
This [video](#) shows the feeding of *Stentor*.

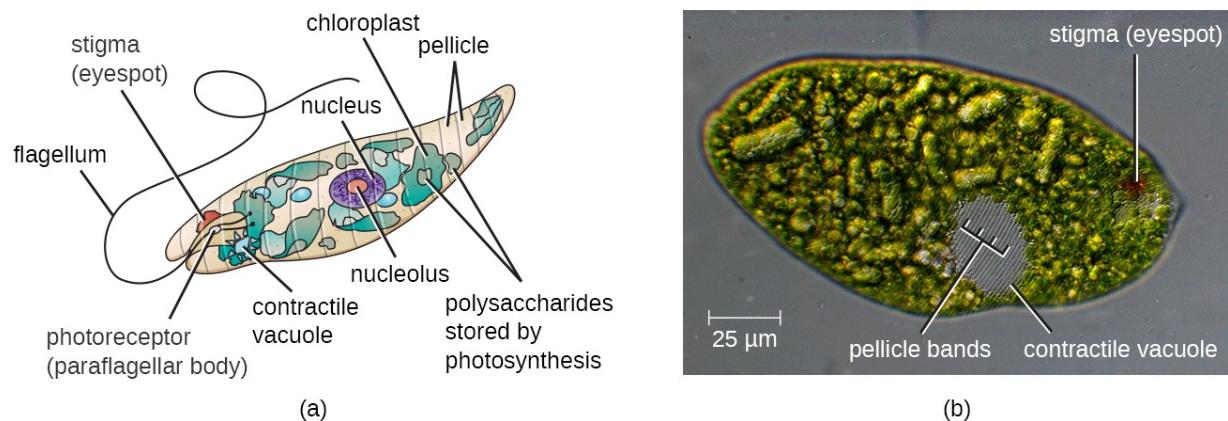
Excavata

The third and final supergroup to be considered in this section is the Excavata, which includes primitive eukaryotes and many parasites with limited metabolic abilities. These organisms have complex cell shapes and structures, often including a depression on the surface of the cell called an excavate. The group Excavata includes the subgroups Fornicata, Parabasalia, and Euglenozoa. The Fornicata lack mitochondria but have flagella. This group includes *Giardia lamblia* (also known as *G. intestinalis* or *G. duodenalis*), a widespread pathogen that causes diarrheal illness and can be spread through cysts from feces that contaminate water supplies ([\[link\]](#)). Parabasalia are frequent animal endosymbionts; they live in the guts of animals like termites and cockroaches. They have basal bodies and modified mitochondria (kinetoplastids). They also have a large, complex cell structure with an undulating membrane and often have many flagella. The trichomonads (a subgroup of the Parabasalia) include pathogens such as *Trichomonas vaginalis*, which causes the human sexually transmitted disease trichomoniasis. Trichomoniasis often does not cause symptoms in men, but men are able to transmit the infection. In women, it causes vaginal discomfort and discharge and may cause complications in pregnancy if left untreated.

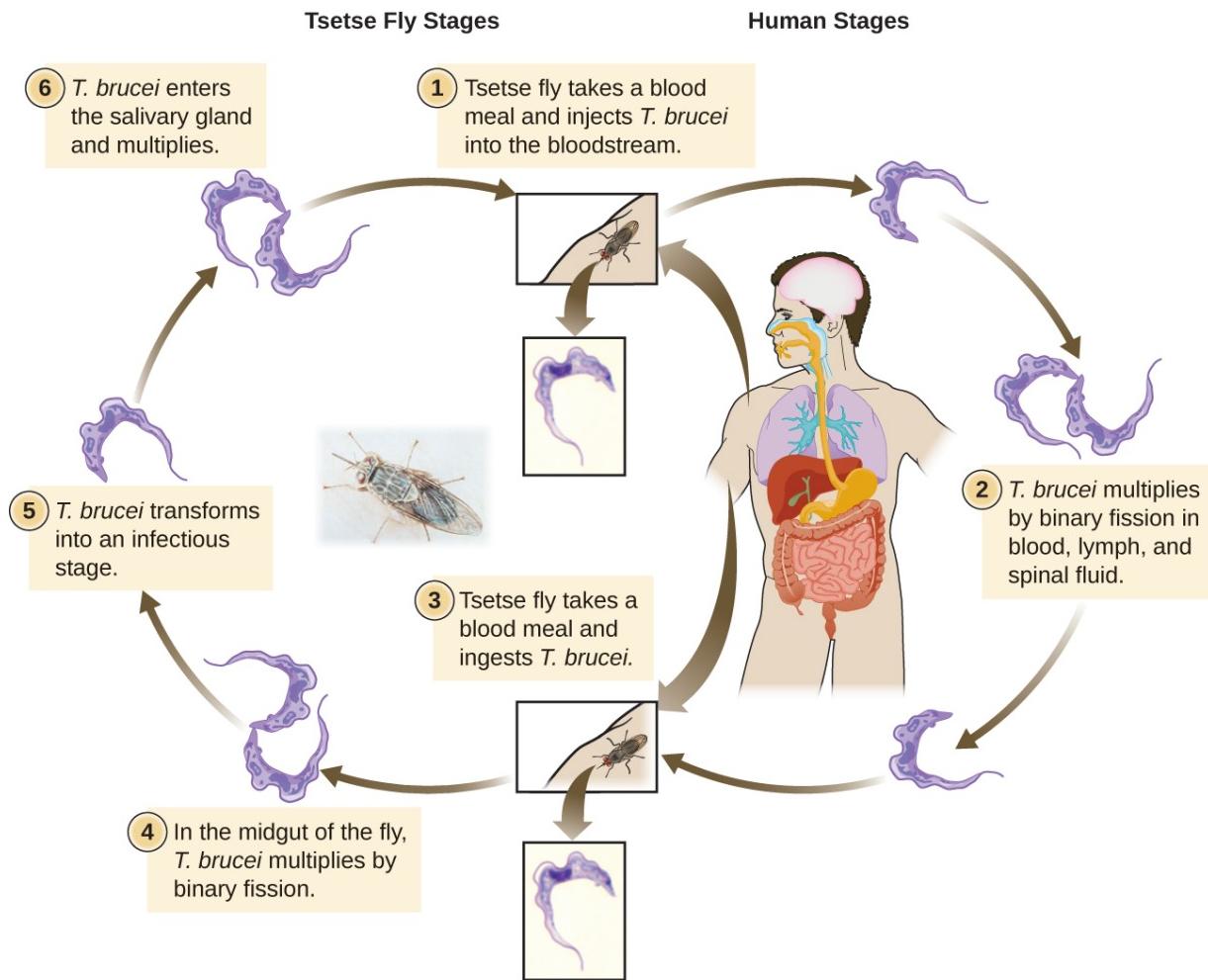
The Euglenozoa are common in the environment and include photosynthetic and nonphotosynthetic species. Members of the genus *Euglena* are typically not pathogenic. Their cells have two flagella, a pellicle, a **stigma** (eyespot) to sense light, and chloroplasts for photosynthesis ([\[link\]](#)). The pellicle of *Euglena* is made of a series of protein bands surrounding the cell; it supports the cell membrane and gives the cell shape.

The Euglenozoa also include the trypanosomes, which are parasitic pathogens. The genus *Trypanosoma* includes *T. brucei*, which causes African trypanosomiasis (African sleeping sickness) and *T. cruzi*, which causes American trypanosomiasis (Chagas disease). These tropical diseases are spread by insect bites. In African sleeping sickness, *T. brucei* colonizes the blood and the brain after being transmitted via the bite of a tsetse fly.

(*Glossina spp.*) ([\[link\]](#)). The early symptoms include confusion, difficulty sleeping, and lack of coordination. Left untreated, it is fatal.



(a) This illustration of a *Euglena* shows the characteristic structures, such as the stigma and flagellum. (b) The pellicle, under the cell membrane, gives the cell its distinctive shape and is visible in this image as delicate parallel striations over the surface of the entire cell (especially visible over the grey contractile vacuole). (credit a: modification of work by Claudio Miklos; credit b: modification of work by David Shykind)



Trypanosoma brucei, the causative agent of African trypanosomiasis, spends part of its life cycle in the tsetse fly and part in humans. (credit “illustration”: modification of work by Centers for Disease Control and Prevention; credit “photo”: DPDx/Centers for Disease Control and Prevention)

Chagas' disease originated and is most common in Latin America. The disease is transmitted by *Triatoma* spp., insects often called “kissing bugs,” and affects either the heart tissue or tissues of the digestive system. Untreated cases can eventually lead to heart failure or significant digestive or neurological disorders.

The genus *Leishmania* includes trypanosomes that cause disfiguring skin disease and sometimes systemic illness as well.

Note:

Neglected Parasites

The Centers for Disease Control and Prevention (CDC) is responsible for identifying public health priorities in the United States and developing strategies to address areas of concern. As part of this mandate, the CDC has officially identified five parasitic diseases it considers to have been neglected (i.e., not adequately studied). These neglected parasitic infections (NPIs) include toxoplasmosis, Chagas disease, toxocariasis (a nematode infection transmitted primarily by infected dogs), cysticercosis (a disease caused by a tissue infection of the tapeworm *Taenia solium*), and trichomoniasis (a sexually transmitted disease caused by the parabasalid *Trichomonas vaginalis*).

The decision to name these specific diseases as NPIs means that the CDC will devote resources toward improving awareness and developing better diagnostic testing and treatment through studies of available data. The CDC may also advise on treatment of these diseases and assist in the distribution of medications that might otherwise be difficult to obtain.

[footnote]

Centers for Disease Control and Prevention. “Neglected Parasitic Infections (NPIs) in the United States.” <http://www.cdc.gov/parasites/npi/>. Last updated July 10, 2014.

Of course, the CDC does not have unlimited resources, so by prioritizing these five diseases, it is effectively deprioritizing others. Given that many Americans have never heard of many of these NPIs, it is fair to ask what criteria the CDC used in prioritizing diseases. According to the CDC, the factors considered were the number of people infected, the severity of the illness, and whether the illness can be treated or prevented. Although several of these NPIs may seem to be more common outside the United States, the CDC argues that many cases in the United States likely go undiagnosed and untreated because so little is known about these diseases.

[footnote]

Centers for Disease Control and Prevention. "Fact Sheet: Neglected Parasitic Infections in the United States."

http://www.cdc.gov/parasites/resources/pdf/npi_factsheet.pdf

What criteria should be considered when prioritizing diseases for purposes of funding or research? Are those identified by the CDC reasonable? What other factors could be considered? Should government agencies like the CDC have the same criteria as private pharmaceutical research labs? What are the ethical implications of deprioritizing other potentially neglected parasitic diseases such as leishmaniasis?

Key Concepts and Summary

- **Protists** are a diverse, **polyphyletic** group of eukaryotic organisms.
- Protists may be unicellular or multicellular. They vary in how they get their nutrition, morphology, method of locomotion, and mode of reproduction.
- Important structures of protists include **contractile vacuoles**, cilia, flagella, **pellicles**, and pseudopodia; some lack organelles such as mitochondria.
- Taxonomy of protists is changing rapidly as relationships are reassessed using newer techniques.
- The protists include important pathogens and parasites.

Multiple Choice

Exercise:

Problem: Which genus includes the causative agent for malaria?

- A. *Euglena*
- B. *Paramecium*
- C. *Plasmodium*
- D. *Trypanosoma*

Solution:

C

Exercise:

Problem:

Which protist is a concern because of its ability to contaminate water supplies and cause diarrheal illness?

- A. *Plasmodium vivax*
 - B. *Toxoplasma gondii*
 - C. *Giardia lamblia*
 - D. *Trichomonas vaginalis*
-

Solution:

C

Fill in the Blank

Exercise:

Problem: The plasma membrane of a protist is called the _____.

Solution:

plasmalemma

Exercise:

Problem:

Animals belong to the same supergroup as the kingdom _____.

Solution:

Fungi

Short Answer

Exercise:

Problem: What are kinetoplastids?

Exercise:

Problem:

Aside from a risk of birth defects, what other effect might a toxoplasmosis infection have?

Exercise:

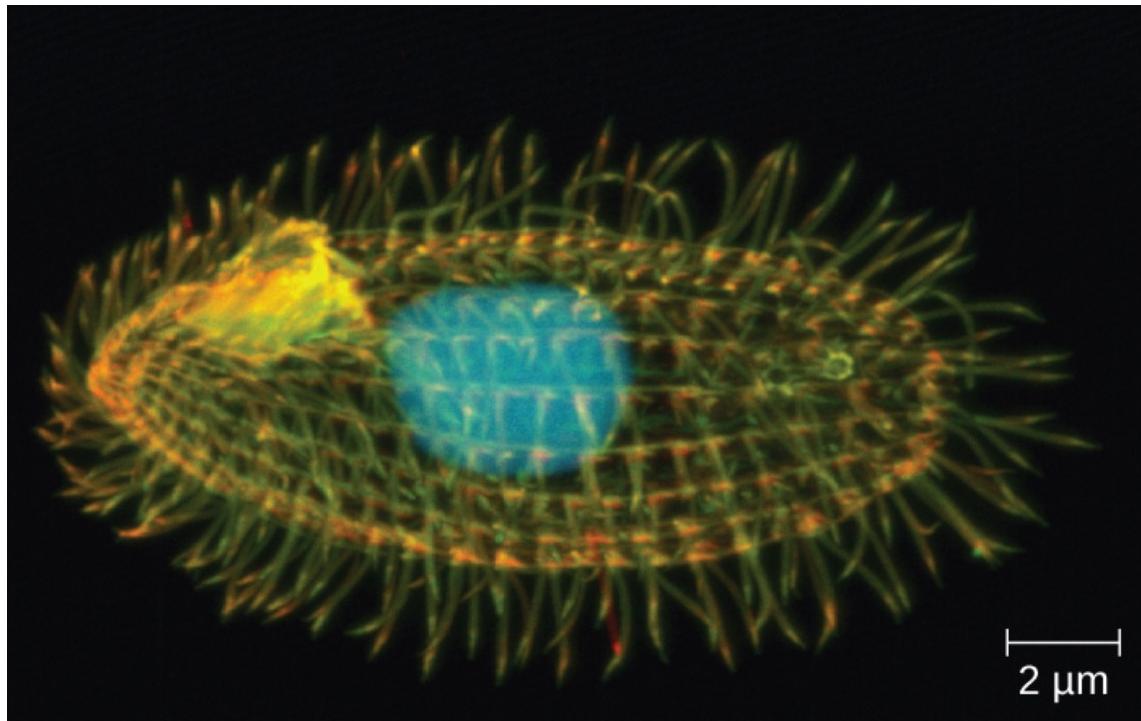
Problem: What is the function of the ciliate macronucleus?

Critical Thinking

Exercise:

Problem: The protist shown has which of the following?

- A. pseudopodia
- B. flagella
- C. a shell
- D. cilia



(credit: modification of work by Richard Robinson)

Exercise:

Problem:

Protist taxonomy has changed greatly in recent years as relationships have been re-examined using newer approaches. How do newer approaches differ from older approaches?

Exercise:

Problem:

What characteristics might make you think a protist could be pathogenic? Are certain nutritional characteristics, methods of locomotion, or morphological differences likely to be associated with the ability to cause disease?

Algae

LEARNING OBJECTIVES

- Explain why algae are included within the discipline of microbiology
- Describe the unique characteristics of algae
- Identify examples of toxin-producing algae
- Compare the major groups of algae in this chapter, and give examples of each
- Classify algal organisms according to major groups

The **algae** are autotrophic protists that can be unicellular or multicellular. These organisms are found in the supergroups Chromalveolata (dinoflagellates, diatoms, golden algae, and brown algae) and Archaeplastida (red algae and green algae). They are important ecologically and environmentally because they are responsible for the production of approximately 70% of the oxygen and organic matter in aquatic environments. Some types of algae, even those that are microscopic, are regularly eaten by humans and other animals. Additionally, algae are the source for **agar**, agarose, and **carrageenan**, solidifying agents used in laboratories and in food production. Although algae are typically not pathogenic, some produce toxins. Harmful **algal blooms**, which occur when algae grow quickly and produce dense populations, can produce high concentrations of toxins that impair liver and nervous-system function in aquatic animals and humans.

Like protozoans, algae often have complex cell structures. For instance, algal cells can have one or more chloroplasts that contain structures called

pyrenoids to synthesize and store starch. The chloroplasts themselves differ in their number of membranes, indicative of secondary or rare tertiary endosymbiotic events. Primary chloroplasts have two membranes—one from the original cyanobacteria that the ancestral eukaryotic cell engulfed, and one from the plasma membrane of the engulfing cell. Chloroplasts in some lineages appear to have resulted from secondary endosymbiosis, in which another cell engulfed a green or red algal cell that already had a primary chloroplast within it. The engulfing cell destroyed everything except the chloroplast and possibly the cell membrane of its original cell, leaving three or four membranes around the chloroplast. Different algal groups have different pigments, which are reflected in common names such as red algae, brown algae, and green algae.

Some algae, the seaweeds, are macroscopic and may be confused with plants. Seaweeds can be red, brown, or green, depending on their photosynthetic pigments. Green algae, in particular, share some important similarities with land plants; however, there are also important distinctions. For example, seaweeds do not have true tissues or organs like plants do. Additionally, seaweeds do not have a waxy cuticle to prevent desiccation. Algae can also be confused with cyanobacteria, photosynthetic bacteria that bear a resemblance to algae; however, cyanobacteria are prokaryotes (see [Nonproteobacteria Gram-negative Bacteria and Phototrophic Bacteria](#)).

Algae have a variety of life cycles. Reproduction may be asexual by mitosis or sexual using gametes.

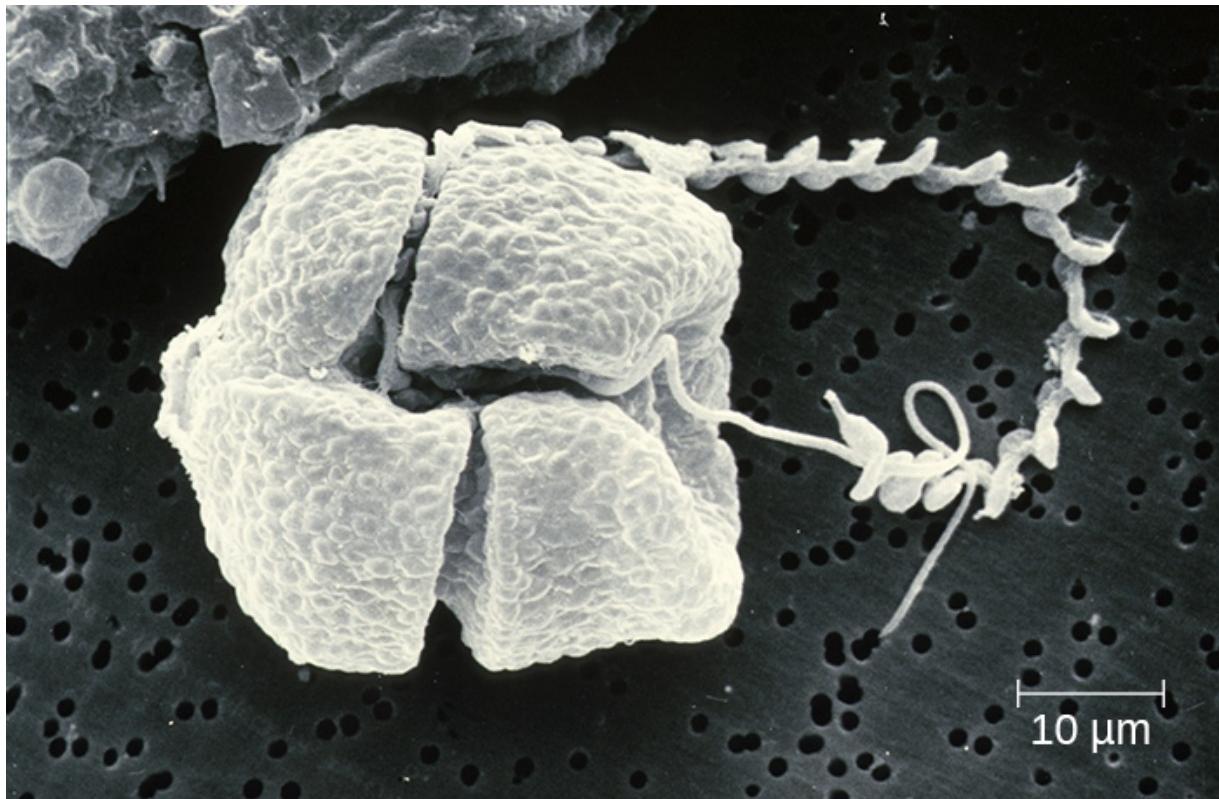
Algal Diversity

Although the algae and protozoa were formerly separated taxonomically, they are now mixed into supergroups. The algae are classified within the Chromalveolata and the Archaeplastida. Although the Euglenozoa (within the supergroup Excavata) include photosynthetic organisms, these are not considered algae because they feed and are motile.

The dinoflagellates and stramenopiles fall within the Chromalveolata. The **dinoflagellates** are mostly marine organisms and are an important component of plankton. They have a variety of nutritional types and may be

phototrophic, heterotrophic, or mixotrophic. Those that are photosynthetic use chlorophyll *a*, chlorophyll *c₂*, and other photosynthetic pigments ([\[link\]](#)). They generally have two flagella, causing them to whirl (in fact, the name dinoflagellate comes from the Greek word for “whirl”: *dini*). Some have cellulose plates forming a hard outer covering, or **theca**, as armor. Additionally, some dinoflagellates produce neurotoxins that can cause paralysis in humans or fish. Exposure can occur through contact with water containing the dinoflagellate toxins or by feeding on organisms that have eaten dinoflagellates.

When a population of dinoflagellates becomes particularly dense, a **red tide** (a type of harmful algal bloom) can occur. Red tides cause harm to marine life and to humans who consume contaminated marine life. Major toxin producers include *Gonyaulax* and *Alexandrium*, both of which cause paralytic shellfish poisoning. Another species, *Pfiesteria piscicida*, is known as a fish killer because, at certain parts of its life cycle, it can produce toxins harmful to fish and it appears to be responsible for a suite of symptoms, including memory loss and confusion, in humans exposed to water containing the species.



The dinoflagellates exhibit great diversity in shape. Many are encased in cellulose armor and have two flagella that fit in grooves between the plates. Movement of these two perpendicular flagella causes a spinning motion. (credit: modification of work by CSIRO)

The **stramenopiles** include the golden algae (Chrysophyta), the brown algae (Phaeophyta), and the **diatoms** (Bacillariophyta). Stramenopiles have chlorophyll *a*, chlorophyll *c₁/c₂*, and fucoxanthin as photosynthetic pigments. Their storage carbohydrate is chrysolaminarin. While some lack cell walls, others have scales. Diatoms have flagella and **frustules**, which are outer cell walls of crystallized silica; their fossilized remains are used to produce diatomaceous earth, which has a range of uses such as filtration and insulation. Additionally, diatoms can reproduce sexually or asexually. One diatom genus, *Pseudo-nitzschia*, is known to be associated with harmful algal blooms.

Brown algae (Phaeophyta) are multicellular marine seaweeds. Some can be extremely large, such as the giant kelp (*Laminaria*). They have leaf-like blades, stalks, and structures called holdfasts that are used to attach to substrate. However, these are not true leaves, stems, or roots ([\[link\]](#)). Their photosynthetic pigments are chlorophyll *a*, chlorophyll *c*, β-carotene, and fucoxanthine. They use laminarin as a storage carbohydrate.

The Archaeplastids include the green algae (Chlorophyta), the red algae (Rhodophyta), another group of green algae (Charophyta), and the land plants. The Charophyta are the most similar to land plants because they share a mechanism of cell division and an important biochemical pathway, among other traits that the other groups do not have. Like land plants, the Charophyta and Chlorophyta have chlorophyll *a* and chlorophyll *b* as photosynthetic pigments, cellulose cell walls, and starch as a carbohydrate storage molecule. *Chlamydomonas* is a green alga that has a single large chloroplast, two flagella, and a stigma (eyespot); it is important in molecular biology research ([\[link\]](#)).

Chlorella is a nonmotile, large, unicellular alga, and *Acetabularia* is an even larger unicellular green alga. The size of these organisms challenges the idea that all cells are small, and they have been used in genetics research since Joachim Hämerling (1901–1980) began to work with them in 1943. *Volvox* is a colonial, unicellular alga ([\[link\]](#)). A larger, multicellular green alga is *Ulva*, also known as the sea lettuce because of its large, edible, green blades. The range of life forms within the Chlorophyta—from unicellular to various levels of coloniality to multicellular forms—has been a useful research model for understanding the evolution of multicellularity. The red algae are mainly multicellular but include some unicellular forms. They have rigid cell walls containing agar or carrageenan, which are useful as food solidifying agents and as a solidifier added to growth media for microbes.



(a)



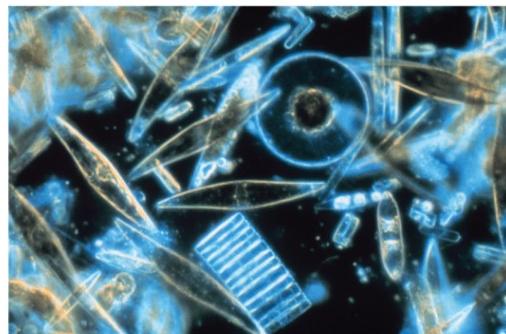
(b)



(c)



(d)



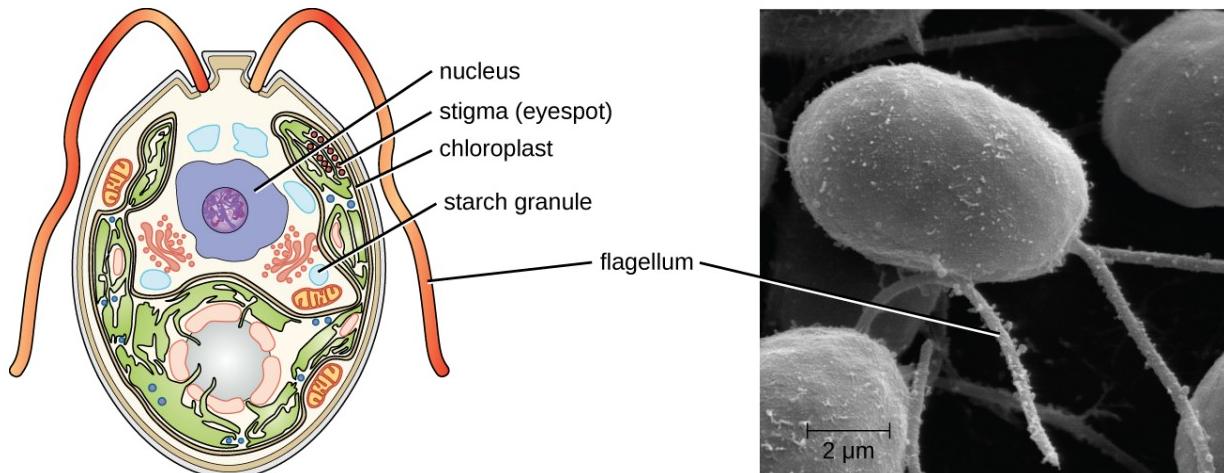
(e)



(f)

(a) These large multicellular kelps are members of the brown algae. Note the “leaves” and “stems” that make them appear similar to green plants. (b) This is a species of red algae that is also multicellular. (c) The green alga *Halimeda incrassata*, shown here growing on the sea floor in shallow water, appears to have plant-like structures, but is not a true plant. (d) Bioluminescence, visible in the cresting wave in this picture, is a phenomenon of certain dinoflagellates. (e) Diatoms (pictured in this micrograph) produce silicaceous tests (skeletons) that

form diatomaceous earths. (f) Colonial green algae, like volvox in these three micrographs, exhibit simple cooperative associations of cells. (credit a, e: modification of work by NOAA; credit b: modification of work by Ed Bierman; credit c: modification of work by James St. John; credit d: modification of work by “catalano82”/Flickr; credit f: modification of work by Dr. Ralf Wagner)



Chlamydomonas is a unicellular green alga.

Note:

- Which groups of algae are associated with harmful algal blooms?

Key Concepts and Summary

- Algae are a diverse group of photosynthetic eukaryotic protists
- Algae may be unicellular or multicellular
- Large, multicellular algae are called seaweeds but are not plants and lack plant-like tissues and organs
- Although algae have little pathogenicity, they may be associated with toxic **algal blooms** that can affect aquatic wildlife and contaminate seafood with toxins that cause paralysis
- Algae are important for producing **agar**, which is used as a solidifying agent in microbiological media, and **carrageenan**, which is used as a solidifying agent

Multiple Choice

Exercise:

Problem:

Which polysaccharide found in red algal cell walls is a useful solidifying agent?

- A. chitin
- B. cellulose
- C. phycoerythrin
- D. agar

Solution:

D

Exercise:

Problem:

Which is the term for the hard outer covering of some dinoflagellates?

- A. theca
- B. thallus
- C. mycelium

D. shell

Solution:

A

Exercise:

Problem: Which protists are associated with red tides?

- A. red algae
 - B. brown algae
 - C. dinoflagellates
 - D. green algae
-

Solution:

C

Fill in the Blank

Exercise:

Problem:

Structures in chloroplasts used to synthesize and store starch are called

_____.

Solution:

pyrenoids

Exercise:

Problem:

Algae with chloroplasts with three or four membranes are a result of

_____.

Solution:

secondary endosymbiosis

Short Answer

Exercise:

Problem: What is a distinctive feature of diatoms?

Exercise:

Problem: Why are algae not considered parasitic?

Exercise:

Problem: Which groups contain the multicellular algae?

Lichens

LEARNING OBJECTIVES

- Explain why lichens are included in the study of microbiology
- Describe the unique characteristics of a lichen and the role of each partner in the symbiotic relationship of a lichen
- Describe ways in which lichens are beneficial to the environment

No one has to worry about getting sick from a lichen infection, but lichens are interesting from a microbiological perspective and they are an important component of most terrestrial ecosystems. Lichens provide opportunities for study of close relationships between unrelated microorganisms. Lichens contribute to soil production by breaking down rock, and they are early colonizers in soilless environments such as lava flows. The cyanobacteria in some lichens can fix nitrogen and act as a nitrogen source in some environments. Lichens are also important soil stabilizers in some desert environments and they are an important winter food source for caribou and reindeer. Finally, lichens produce compounds that have antibacterial effects, and further research may discover compounds that are medically useful to humans.

Characteristics

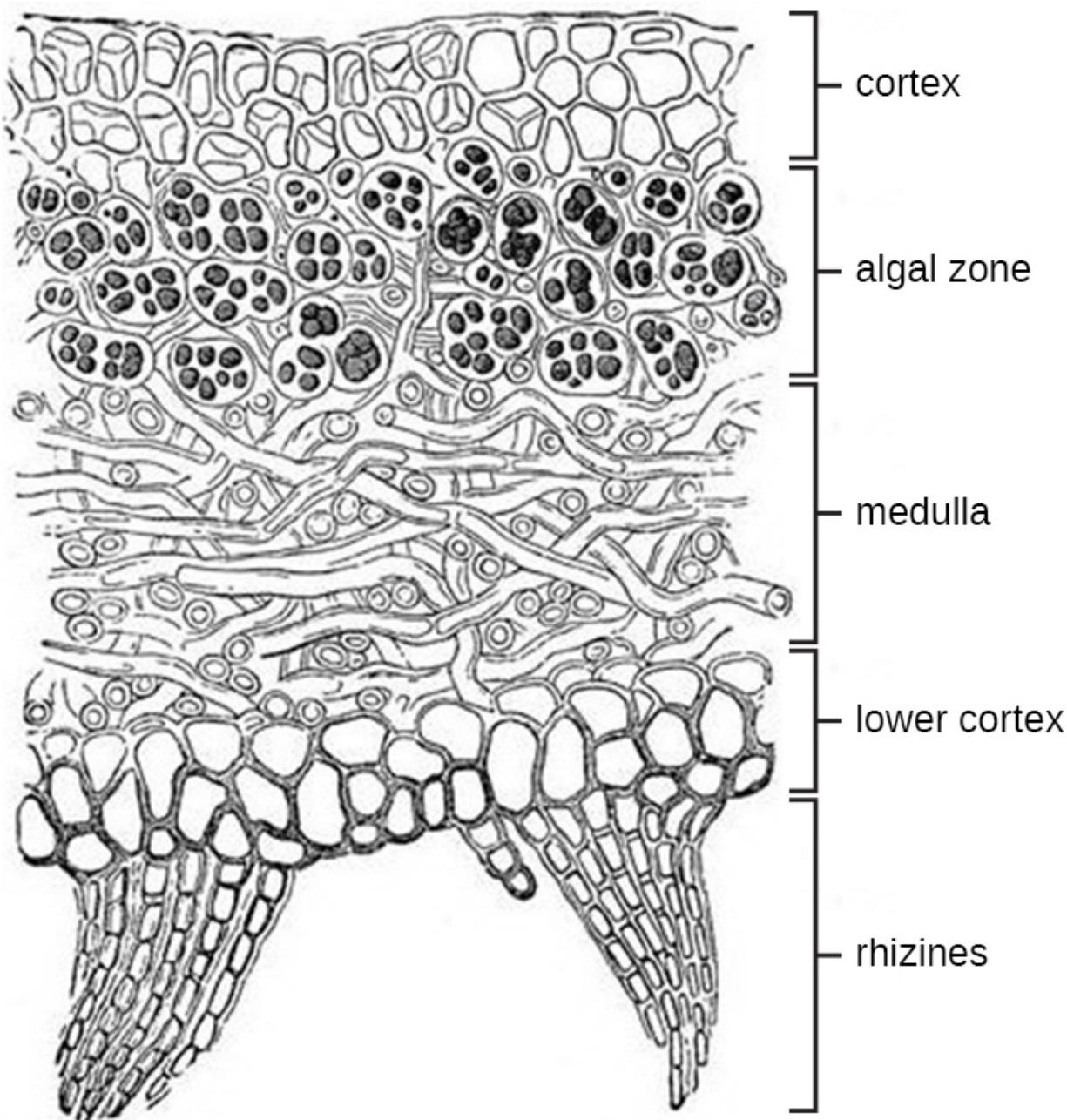
A **lichen** is a combination of two organisms, a green alga or cyanobacterium and an ascomycete fungus, living in a symbiotic relationship. Whereas algae normally grow only in aquatic or extremely

moist environments, lichens can potentially be found on almost any surface (especially rocks) or as **epiphytes** (meaning that they grow on other plants).

In some ways, the symbiotic relationship between lichens and algae seems like a mutualism (a relationship in which both organisms benefit). The fungus can obtain photosynthates from the algae or cyanobacterium and the algae or cyanobacterium can grow in a drier environment than it could otherwise tolerate. However, most scientists consider this symbiotic relationship to be a controlled parasitism (a relationship in which one organism benefits and the other is harmed) because the photosynthetic organism grows less well than it would without the fungus. It is important to note that such symbiotic interactions fall along a continuum between conflict and cooperation.

Lichens are slow growing and can live for centuries. They have been used in foods and to extract chemicals as dyes or antimicrobial substances. Some are very sensitive to pollution and have been used as environmental indicators.

Lichens have a body called a thallus, an outer, tightly packed fungal layer called a **cortex**, and an inner, loosely packed fungal layer called a **medulla** ([\[link\]](#)). Lichens use hyphal bundles called **rhizines** to attach to the substrate.



This cross-section of a lichen thallus shows its various components. The upper cortex of fungal hyphae provides protection. Photosynthesis occurs in the algal zone. The medulla consists of fungal hyphae. The lower cortex also provides protection. The rhizines anchor the thallus to the substrate.

Lichen Diversity

Lichens are classified as fungi and the fungal partners belong to the Ascomycota and Basidiomycota. Lichens can also be grouped into types based on their morphology. There are three major types of lichens, although other types exist as well. Lichens that are tightly attached to the substrate, giving them a crusty appearance, are called **crustose lichens**. Those that have leaf-like lobes are **foliose lichens**; they may only be attached at one point in the growth form, and they also have a second cortex below the medulla. Finally, **fruticose lichens** have rounded structures and an overall branched appearance. [\[link\]](#) shows an example of each of the forms of lichens.



(a)



(b)



(c)

Examples of the three types of lichens are shown here. (a) This is a crustose lichen found mostly on marine rocks, *Caloplaca marina*. (b) This is a foliose lichen, *Flavoparmelia caperata*. (c) This is a fruticose lichen, *Letharia vulpina*, which is sufficiently poisonous that it was once used to make arrowheads. (credit b, c: modification of work by Jason Hollinger)

Note:

- What types of organisms are found in lichens?
- What are the three growth forms of lichens?

Note:

Resolution

Sarah's mother asks the doctor what she should do if the cream prescribed for Sarah's ringworm does not work. The doctor explains that ringworm is a general term for a condition caused by multiple species. The first step is to take a scraping for examination under the microscope, which the doctor has already done. He explains that he has identified the infection as a fungus, and that the antifungal cream works against the most common fungi associated with ringworm. However, the cream may not work against some species of fungus. If the cream is not working after a couple of weeks, Sarah should come in for another visit, at which time the doctor will take steps to identify the species of the fungus.

Positive identification of dermatophytes requires culturing. For this purpose, Sabouraud's agar may be used. In the case of Sarah's infection, which cleared up within 2 weeks of treatment, the culture would have a granular texture and would appear pale pink on top and red underneath. These features suggest that the fungus is *Trichophyton rubrum*, a common cause of ringworm.

Go back to the [previous](#) Clinical Focus box.

Key Concepts and Summary

- **Lichens** are a symbiotic association between a fungus and an algae or a cyanobacterium
- The symbiotic association found in lichens is currently considered to be a controlled **parasitism**, in which the fungus benefits and the algae or cyanobacterium is harmed
- Lichens are slow growing and can live for centuries in a variety of habitats

- Lichens are environmentally important, helping to create soil, providing food, and acting as indicators of air pollution

Multiple Choice

Exercise:

Problem:

You encounter a lichen with leafy structures. Which term describes this lichen?

- A. crustose
- B. foliose
- C. fruticose
- D. agarose

Solution:

B

Exercise:

Problem:

Which of the following is the term for the outer layer of a lichen?

- A. the cortex
- B. the medulla
- C. the thallus
- D. the theca

Solution:

A

Exercise:

Problem: The fungus in a lichen is which of the following?

- A. a basidiomycete
 - B. an ascomycete
 - C. a zygomycete
 - D. an apicomplexan
-

Solution:

B

Short Answer

Exercise:

Problem:

What are three ways that lichens are environmentally valuable?

Fungi

LEARNING OBJECTIVES

- Explain why the study of fungi such as yeast and molds is within the discipline of microbiology
- Describe the unique characteristics of fungi
- Describe examples of asexual and sexual reproduction of fungi
- Compare the major groups of fungi in this chapter, and give examples of each
- Identify examples of the primary causes of infections due to yeasts and molds
- Identify examples of toxin-producing fungi
- Classify fungal organisms according to major groups

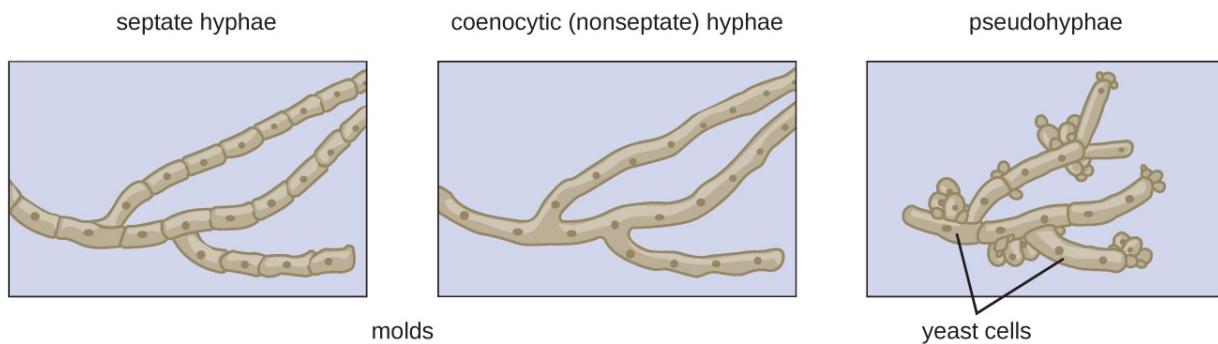
The fungi comprise a diverse group of organisms that are heterotrophic and typically saprozoic. In addition to the well-known macroscopic fungi (such as mushrooms and molds), many unicellular yeasts and spores of macroscopic fungi are microscopic. For this reason, fungi are included within the field of microbiology.

Fungi are important to humans in a variety of ways. Both microscopic and macroscopic fungi have medical relevance, with some pathogenic species that can cause **mycoses** (illnesses caused by fungi). Some pathogenic fungi are opportunistic, meaning that they mainly cause infections when the host's immune defenses are compromised and do not normally cause illness in healthy individuals. Fungi are important in other ways. They act as decomposers in the environment, and they are critical for the production of

certain foods such as cheeses. Fungi are also major sources of antibiotics, such as penicillin from the fungus *Penicillium*.

Characteristics of Fungi

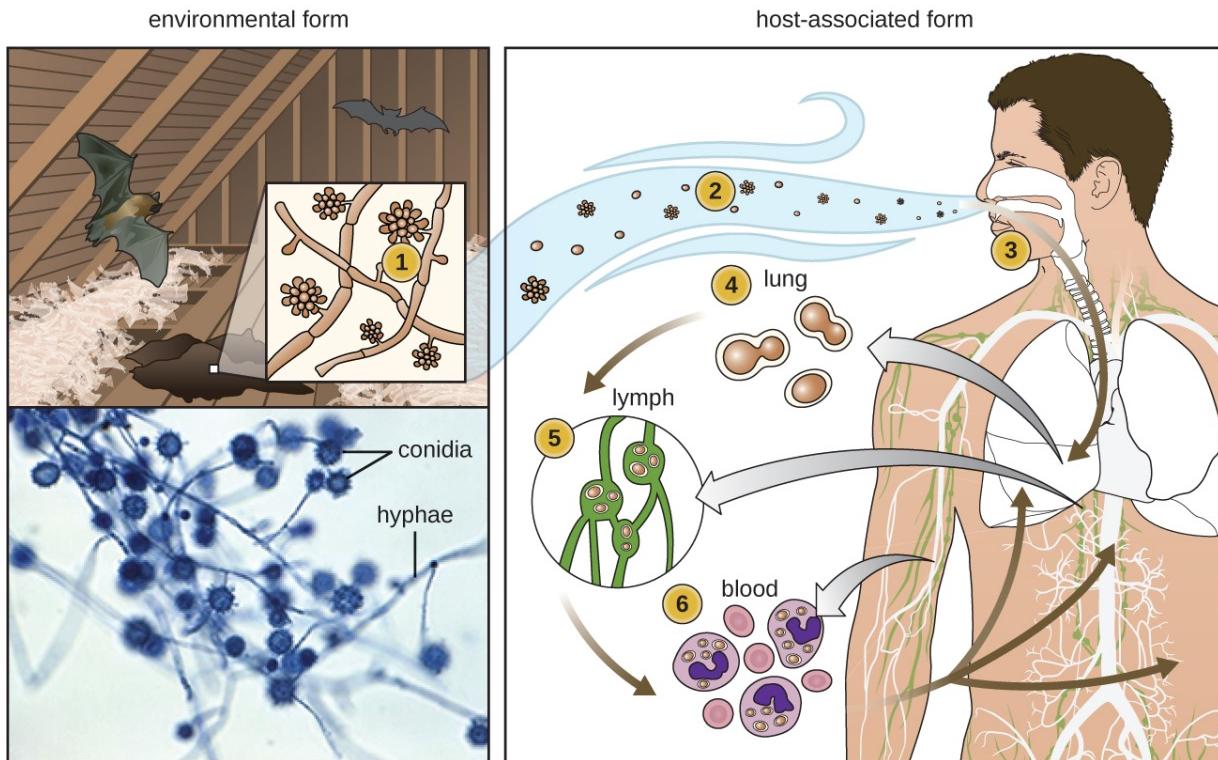
Fungi have well-defined characteristics that set them apart from other organisms. Most multicellular fungal bodies, commonly called molds, are made up of filaments called **hyphae**. Hyphae can form a tangled network called a **mycelium** and form the **thallus** (body) of fleshy fungi. Hyphae that have walls between the cells are called **septate hyphae**; hyphae that lack walls and cell membranes between the cells are called nonseptate or **coenocytic hyphae**. ([\[link\]](#)).



Multicellular fungi (molds) form hyphae, which may be septate or nonseptate. Unicellular fungi (yeasts) cells form pseudohyphae from individual yeast cells.

In contrast to molds, yeasts are unicellular fungi. The **budding yeasts** reproduce asexually by budding off a smaller daughter cell; the resulting cells may sometimes stick together as a short chain or **pseudohypha** ([\[link\]](#)). *Candida albicans* is a common yeast that forms pseudohyphae; it is associated with various infections in humans, including vaginal yeast infections, oral thrush, and candidiasis of the skin.

Some fungi are dimorphic, having more than one appearance during their life cycle. These **dimorphic fungi** may be able to appear as yeasts or molds, which can be important for infectivity. They are capable of changing their appearance in response to environmental changes such as nutrient availability or fluctuations in temperature, growing as a mold, for example, at 25 °C (77 °F), and as yeast cells at 37 °C (98.6 °F). This ability helps dimorphic fungi to survive in diverse environments. *Histoplasma capsulatum*, the pathogen that causes histoplasmosis, a lung infection, is an example of a dimorphic fungus ([\[link\]](#)).



Histoplasma capsulatum is a dimorphic fungus that grows in soil exposed to bird feces or bat feces (guano) (top left). It can change forms to survive at different temperatures. In the outdoors, it typically grows as a mycelium (as shown in the micrograph, bottom left), but when the spores are inhaled (right), it responds to the high internal temperature of the body (37 °C [98.6 °F]) by turning into a yeast that can multiply in the lungs, causing the chronic lung disease

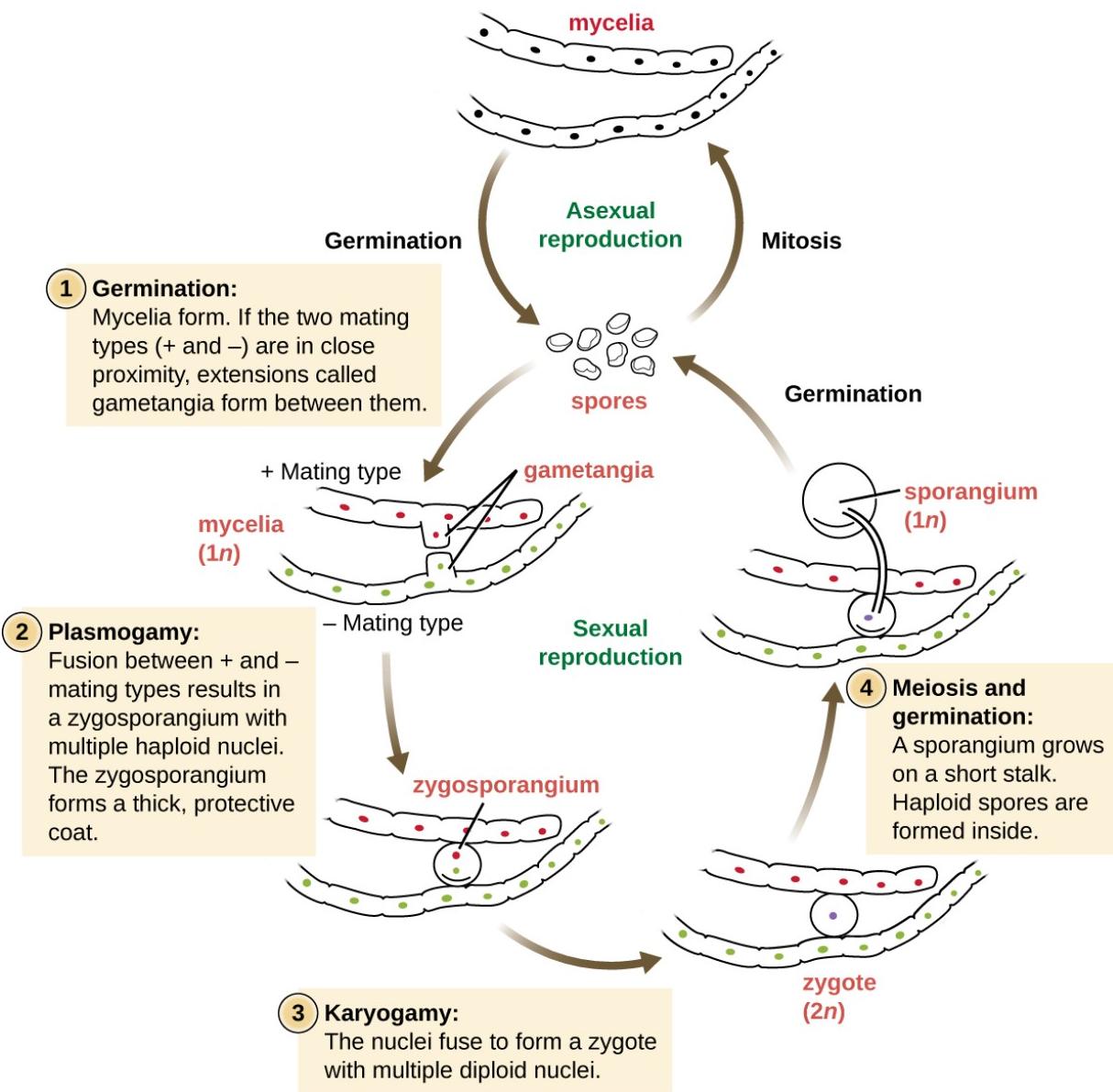
histoplasmosis. (credit: modification of work by Centers for Disease Control and Prevention)

There are notable unique features in fungal cell walls and membranes. Fungal cell walls contain **chitin**, as opposed to the cellulose found in the cell walls of plants and many protists. Additionally, whereas animals have cholesterol in their cell membranes, fungal cell membranes have different sterols called ergosterols. Ergosterols are often exploited as targets for antifungal drugs.

Fungal life cycles are unique and complex. Fungi reproduce sexually either through cross- or self-fertilization. Haploid fungi form hyphae that have gametes at the tips. Two different mating types (represented as “+ type” and “– type”) are involved. The cytoplasms of the + and – type gametes fuse (in an event called plasmogamy), producing a cell with two distinct nuclei (a **dikaryotic** cell). Later, the nuclei fuse (in an event called karyogamy) to create a diploid zygote. The zygote undergoes meiosis to form **spores** that germinate to start the haploid stage, which eventually creates more haploid mycelia ([\[link\]](#)). Depending on the taxonomic group, these sexually produced spores are known as zygosporcs (in Zygomycota), ascospores (in Ascomycota), or basidiospores (in Basidiomycota) ([\[link\]](#)).

Fungi may also exhibit asexual reproduction by mitosis, mitosis with budding, fragmentation of hyphae, and formation of asexual spores by mitosis. These spores are specialized cells that, depending on the organism, may have unique characteristics for survival, reproduction, and dispersal. Fungi exhibit several types of asexual spores and these can be important in classification.

Zygomycete Life Cycle



Zygomycetes have sexual and asexual life cycles. In the sexual life cycle, + and – mating types conjugate to form a zygosporangium.



These images show asexually produced spores. (a) This brightfield micrograph shows the release of spores from a sporangium at the end of a hypha called a sporangiophore. The organism is a *Mucor* sp. fungus, a mold often found indoors. (b) Sporangia grow at the ends of stalks, which appear as the white fuzz seen on this bread mold, *Rhizopus stolonifer*. The tips of bread mold are the dark, spore-containing sporangia. (credit a: modification of work by Centers for Disease Control and Prevention; credit b right: modification of work by “Andrew”/Flickr)

Note:

- Is a dimorphic fungus a yeast or a mold? Explain.

Fungal Diversity

The fungi are very diverse, comprising seven major groups. Not all of the seven groups contain pathogens. Some of these groups are generally associated with plants and include plant pathogens. For example, Urediniomycetes and Ustilaginomycetes include the plant rusts and smuts, respectively. These form reddish or dark masses, respectively, on plants as rusts (red) or smuts (dark). Some species have substantial economic impact

because of their ability to reduce crop yields. Glomeromycota includes the mycorrhizal fungi, important symbionts with plant roots that can promote plant growth by acting like an extended root system. The Glomeromycota are obligate symbionts, meaning that they can only survive when associated with plant roots; the fungi receive carbohydrates from the plant and the plant benefits from the increased ability to take up nutrients and minerals from the soil. The Chytridiomycetes (chytrids) are small fungi, but are extremely ecologically important. Chytrids are generally aquatic and have flagellated, motile gametes; specific types are implicated in amphibian declines around the world. Because of their medical importance, we will focus on Zygomycota, Ascomycota, Basidiomycota, and Microsporidia. [\[link\]](#) summarizes the characteristics of these medically important groups of fungi.

The Zygomycota (zygomycetes) are mainly saprophytes with coenocytic hyphae and haploid nuclei. They use sporangiospores for asexual reproduction. The group name comes from the **zygospores** that they use for sexual reproduction ([\[link\]](#)), which have hard walls formed from the fusion of reproductive cells from two individuals. Zygomycetes are important for food science and as crop pathogens. One example is *Rhizopus stolonifer* ([\[link\]](#)), an important bread mold that also causes rice seedling blight. *Mucor* is a genus of fungi that can potentially cause necrotizing infections in humans, although most species are intolerant of temperatures found in mammalian bodies ([\[link\]](#)).

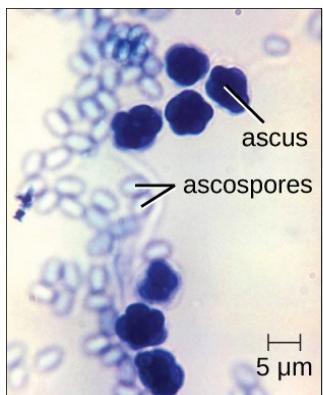
The Ascomycota include fungi that are used as food (edible mushrooms, morels, and truffles), others that are common causes of food spoilage (bread molds and plant pathogens), and still others that are human pathogens. Ascomycota may have septate hyphae and cup-shaped fruiting bodies called **ascocarps**. Some genera of Ascomycota use sexually produced **ascospores** as well as asexual spores called **conidia**, but sexual phases have not been discovered or described for others. Some produce an **ascus** containing ascospores within an ascocarp ([\[link\]](#)).

Examples of the Ascomycota include several bread molds and minor pathogens, as well as species capable of causing more serious mycoses. Species in the genus *Aspergillus* are important causes of allergy and

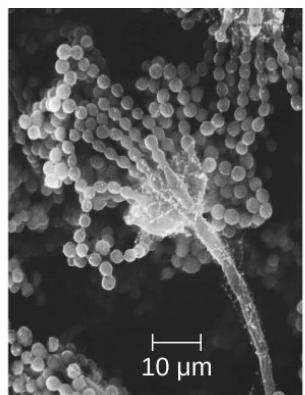
infection, and are useful in research and in the production of certain fermented alcoholic beverages such as Japanese *sake*. The fungus *Aspergillus flavus*, a contaminant of nuts and stored grains, produces an **aflatoxin** that is both a toxin and the most potent known natural carcinogen. *Neurospora crassa* is of particular use in genetics research because the spores produced by meiosis are kept inside the ascus in a row that reflects the cell divisions that produced them, giving a direct view of segregation and assortment of genes ([\[link\]](#)). *Penicillium* produces the antibiotic penicillin ([\[link\]](#)).

Many species of ascomycetes are medically important. A large number of species in the genera *Trichophyton*, *Microsporum*, and *Epidermophyton* are dermatophytes, pathogenic fungi capable of causing skin infections such as athlete's foot, jock itch, and ringworm. *Blastomyces dermatitidis* is a dimorphic fungus that can cause blastomycosis, a respiratory infection that, if left untreated, can become disseminated to other body sites, sometimes leading to death. Another important respiratory pathogen is the dimorphic fungus *Histoplasma capsulatum* ([\[link\]](#)), which is associated with birds and bats in the Ohio and Mississippi river valleys. *Coccidioides immitis* causes the serious lung disease Valley fever. *Candida albicans*, the most common cause of vaginal and other yeast infections, is also an ascomycete fungus; it is a part of the normal microbiota of the skin, intestine, genital tract, and ear ([\[link\]](#)). Ascomycetes also cause plant diseases, including ergot infections, Dutch elm disease, and powdery mildews.

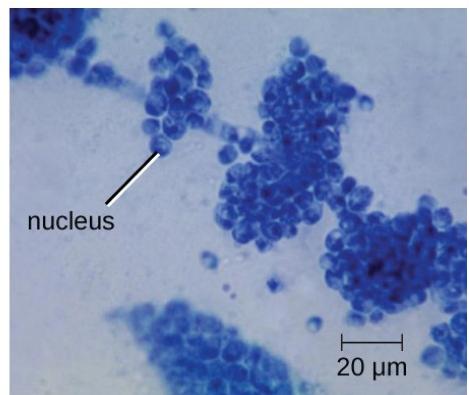
Saccharomyces yeasts, including the baker's yeast *S. cerevisiae*, are unicellular ascomycetes with haploid and diploid stages ([\[link\]](#)). This and other *Saccharomyces* species are used for brewing beer.



(a)

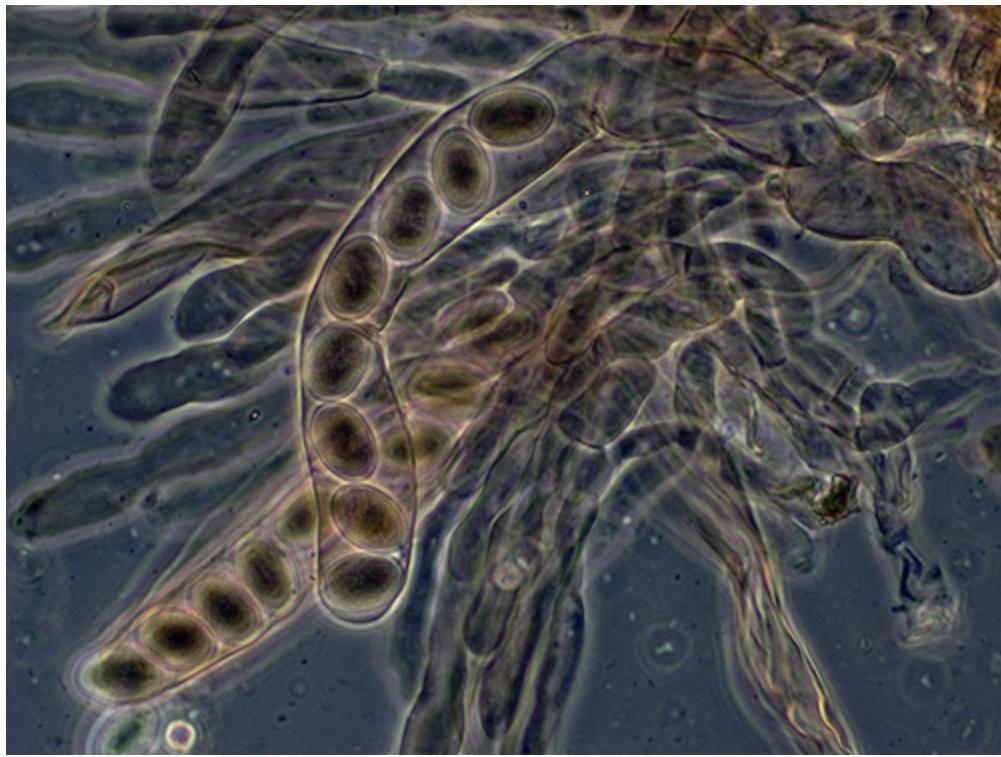


(b)



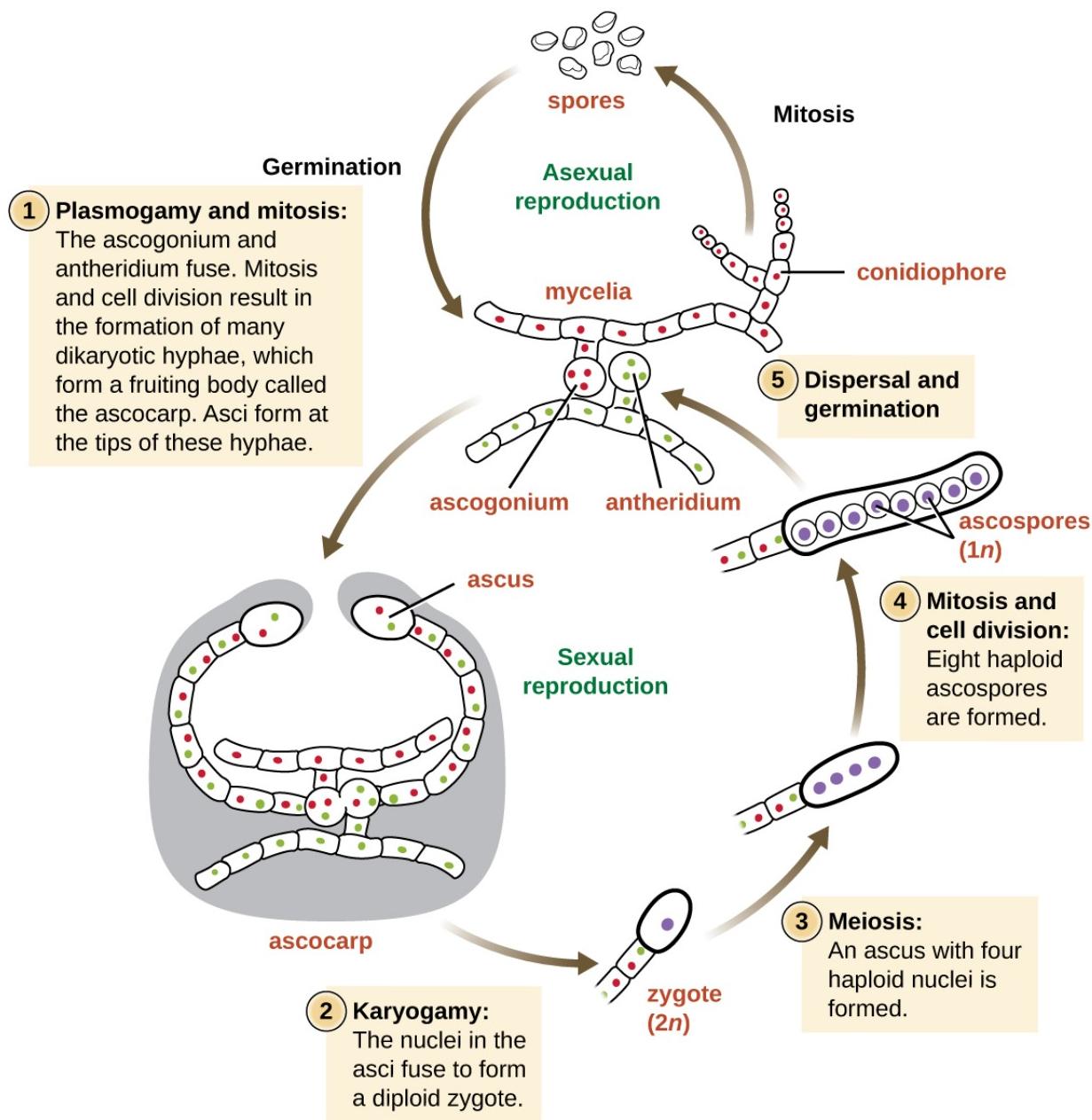
(c)

(a) This brightfield micrograph shows ascospores being released from asci in the fungus *Talaromyces flavus* var. *flavus*. (b) This electron micrograph shows the conidia (spores) borne on the conidiophore of *Aspergillus*, a type of toxic fungus found mostly in soil and plants. (c) This brightfield micrograph shows the yeast *Candida albicans*, the causative agent of candidiasis and thrush. (credit a, b, c: modification of work by Centers for Disease Control and Prevention)



These ascospores, lined up within an ascus, are produced sexually. (credit: Peter G. Werner)

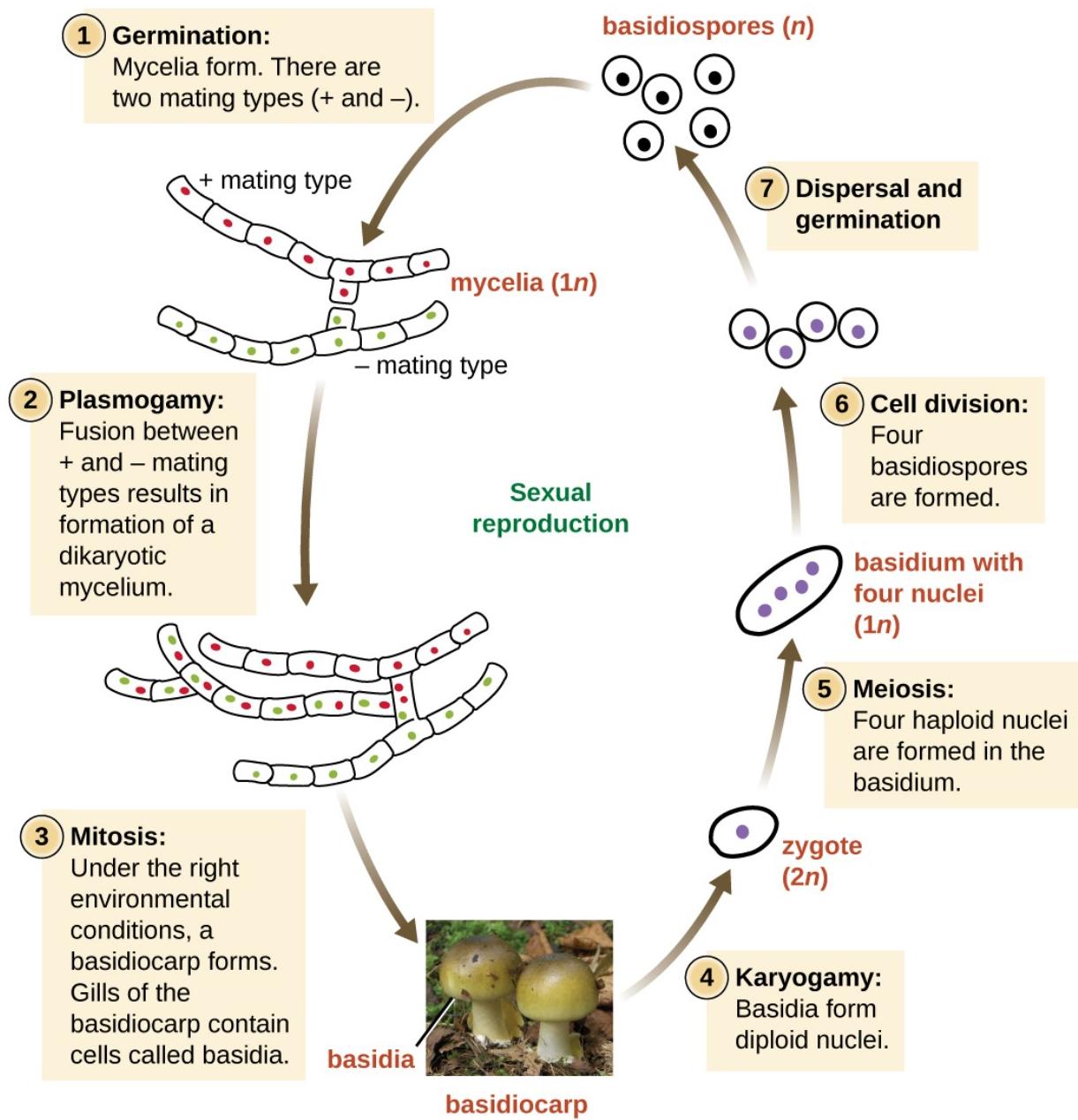
Ascomycete Life Cycle



The life cycle of an ascomycete is characterized by the production of ascii during the sexual phase. The haploid phase is the predominant phase of the life cycle.

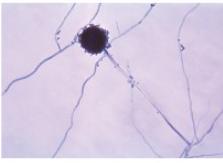
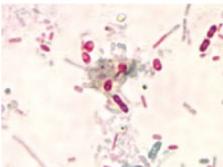
The Basidiomycota (basidiomycetes) are fungi that have **basidia** (club-shaped structures) that produce **basidiospores** (spores produced through budding) within fruiting bodies called **basidiocarps** ([\[link\]](#)). They are important as decomposers and as food. This group includes rusts, stinkhorns, puffballs, and mushrooms. Several species are of particular importance. *Cryptococcus neoformans*, a fungus commonly found as a yeast in the environment, can cause serious lung infections when inhaled by individuals with weakened immune systems. The edible meadow mushroom, *Agricus campestris*, is a basidiomycete, as is the poisonous mushroom *Amanita phalloides*, known as the death cap. The deadly toxins produced by *A. phalloides* have been used to study transcription.

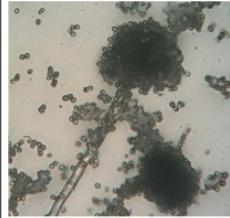
Basidiomycete Life Cycle



The life cycle of a basidiomycete alternates a haploid generation with a prolonged stage in which two nuclei (dikaryon) are present in the hyphae.

Finally, the **Microsporidia** are unicellular fungi that are obligate intracellular parasites. They lack mitochondria, peroxisomes, and centrioles, but their spores release a unique **polar tubule** that pierces the host cell membrane to allow the fungus to gain entry into the cell. A number of microsporidia are human pathogens, and infections with microsporidia are called microsporidiosis. One pathogenic species is *Enterocystozoan bieneusi*, which can cause symptoms such as diarrhea, cholecystitis (inflammation of the gall bladder), and in rare cases, respiratory illness.

Select Groups of Fungi				
Group	Characteristics	Examples	Medically Important Species	Image
Ascomycota	Septate hyphae Ascus with ascospores in ascocarp Conidiospores	Cup fungi Edible mushrooms Morels Truffles <i>Neurospora</i> <i>Penicillium</i>	<i>Aspergillus</i> spp. <i>Trichophyton</i> spp. <i>Microsporum</i> spp. <i>Epidermophyton</i> spp. <i>Blastomyces dermatitidis</i> <i>Histoplasma capsulatum</i>	 <i>Aspergillus niger</i>
Basidiomycota	Basidia produce basidiospores in a basidiocarp	Club fungi Rusts Stinkhorns Puffballs Mushrooms <i>Cryptococcus neoformans</i> <i>Amanita phalloides</i>	<i>Cryptococcus neoformans</i>	 <i>Amanita phalloides</i>
Microsporidia	Lack mitochondria, peroxisomes, centrioles Spores produce	<i>Enterocystozoan bieneusi</i>	<i>Enterocystozoan bieneusi</i>	

	a polar tube			
Zygomycota	Mainly saprophytes Coenocytic hyphae Haploid nuclei Zygosores	<i>Rhizopus stolonifera</i>	<i>Mucor</i> spp.	 <i>Rhizopus</i> sp.

(credit “Ascomycota”: modification of work by Dr. Lucille Georg, Centers for Disease Control and Prevention; credit “Microsporidia”: modification of work by Centers for Disease Control and Prevention)

Note:

- Which group of fungi appears to be associated with the greatest number of human diseases?

Note:

Eukaryotic Pathogens in Eukaryotic Hosts

When we think about antimicrobial medications, antibiotics such as penicillin often come to mind. Penicillin and related antibiotics interfere with the synthesis of peptidoglycan cell walls, which effectively targets bacterial cells. These antibiotics are useful because humans (like all eukaryotes) do not have peptidoglycan cell walls.

Developing medications that are effective against eukaryotic cells but not harmful to human cells is more difficult. Despite huge morphological differences, the cells of humans, fungi, and protists are similar in terms of

their ribosomes, cytoskeletons, and cell membranes. As a result, it is more challenging to develop medications that target protozoans and fungi in the same way that antibiotics target prokaryotes.

Fungicides have relatively limited modes of action. Because fungi have ergosterols (instead of cholesterol) in their cell membranes, the different enzymes involved in sterol production can be a target of some medications. The azole and morpholine fungicides interfere with the synthesis of membrane sterols. These are used widely in agriculture (fenpropimorph) and clinically (e.g., miconazole). Some antifungal medications target the chitin cell walls of fungi. Despite the success of these compounds in targeting fungi, antifungal medications for systemic infections still tend to have more toxic side effects than antibiotics for bacteria.

Note:

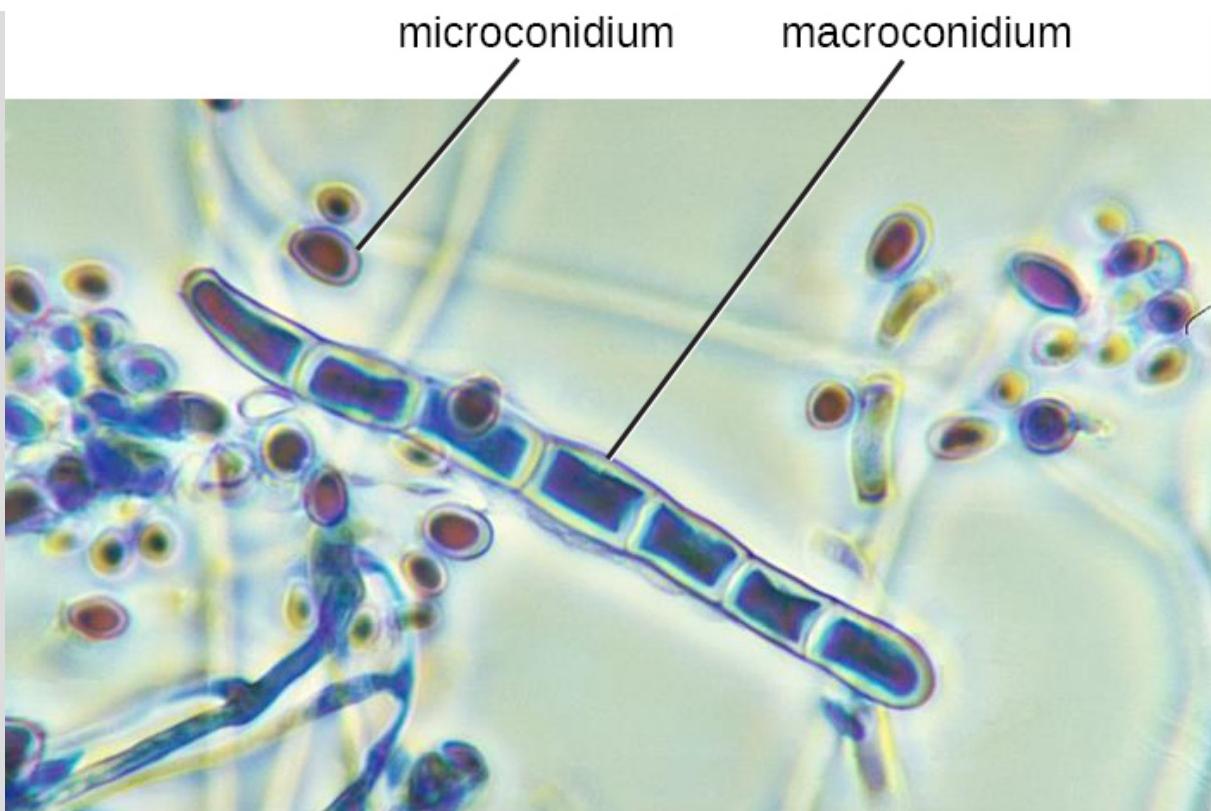
Part 3

Sarah is relieved the ringworm is not an actual worm, but wants to know what it really is. The physician explains that ringworm is a fungus. He tells her that she will not see mushrooms popping out of her skin, because this fungus is more like the invisible part of a mushroom that hides in the soil. He reassures her that they are going to get the fungus out of her too.

The doctor cleans and then carefully scrapes the lesion to place a specimen on a slide. By looking at it under a microscope, the physician is able to confirm that a fungal infection is responsible for Sarah's lesion. In [link], it is possible to see macro- and microconidia in *Trichophyton rubrum*. Cell walls are also visible. Even if the pathogen resembled a helminth under the microscope, the presence of cell walls would rule out the possibility because animal cells lack cell walls.

The doctor prescribes an antifungal cream for Sarah's mother to apply to the ringworm. Sarah's mother asks, "What should we do if it doesn't go away?"

- Can all forms of ringworm be treated with the same antifungal medication?



This micrograph shows hyphae (macroconidium) and microconidia of *Trichophyton rubrum*, a dermatophyte responsible for fungal infections of the skin. (credit: modification of work by Centers for Disease Control and Prevention)

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

Key Concepts and Summary

- The fungi include diverse saprotrophic eukaryotic organisms with chitin cell walls
- Fungi can be unicellular or multicellular; some (like yeast) and fungal spores are microscopic, whereas some are large and conspicuous
- Reproductive types are important in distinguishing fungal groups

- Medically important species exist in the four fungal groups Zygomycota, Ascomycota, Basidiomycota, and Microsporidia
- Members of Zygomycota, Ascomycota, and Basidiomycota produce deadly toxins
- Important differences in fungal cells, such as ergosterols in fungal membranes, can be targets for antifungal medications, but similarities between human and fungal cells make it difficult to find targets for medications and these medications often have toxic adverse effects

Multiple Choice

Exercise:

Problem: Mushrooms are a type of which of the following?

- A. conidia
- B. ascus
- C. polar tubule
- D. basidiocarp

Solution:

D

Exercise:

Problem:

Which of the following is the most common cause of human yeast infections?

- A. *Candida albicans*
- B. *Blastomyces dermatitidis*
- C. *Cryptococcus neoformans*
- D. *Aspergillus fumigatus*

Solution:

A

Exercise:

Problem:

Which of the following is an ascomycete fungus associated with bat droppings that can cause a respiratory infection if inhaled?

- A. *Candida albicans*
 - B. *Histoplasma capsulatum*
 - C. *Rhizopus stolonifera*
 - D. *Trichophyton rubrum*
-

Solution:

B

Fill in the Blank

Exercise:

Problem: Nonseptate hyphae are also called _____.

Solution:

coenocytic

Exercise:

Problem: Unicellular fungi are called _____.

Solution:

yeasts

Exercise:

Problem:

Some fungi have proven medically useful because they can be used to produce _____.

Solution:

antibiotics

Short Answer

Exercise:

Problem:

Which genera of fungi are common dermatophytes (fungi that cause skin infections)?

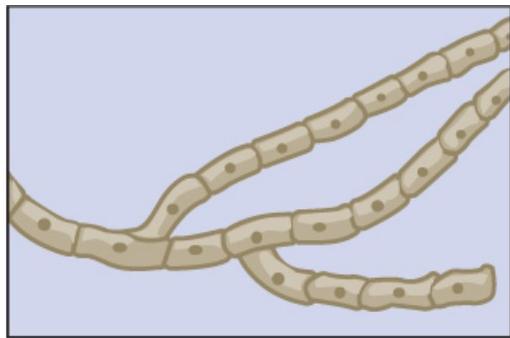
Exercise:

Problem: What is a dikaryotic cell?

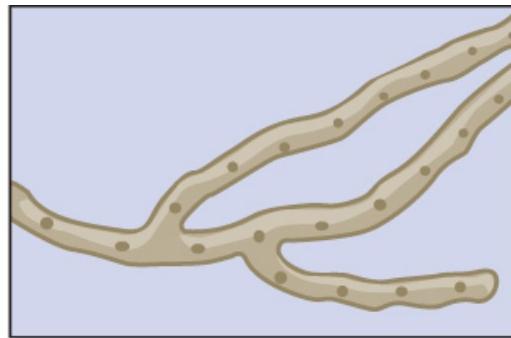
Critical Thinking

Exercise:

Problem: Which of the drawings shows septate hyphae?



A



B

Exercise:

Problem:

Explain the benefit of research into the pathways involved in the synthesis of chitin in fungi.

Helminths

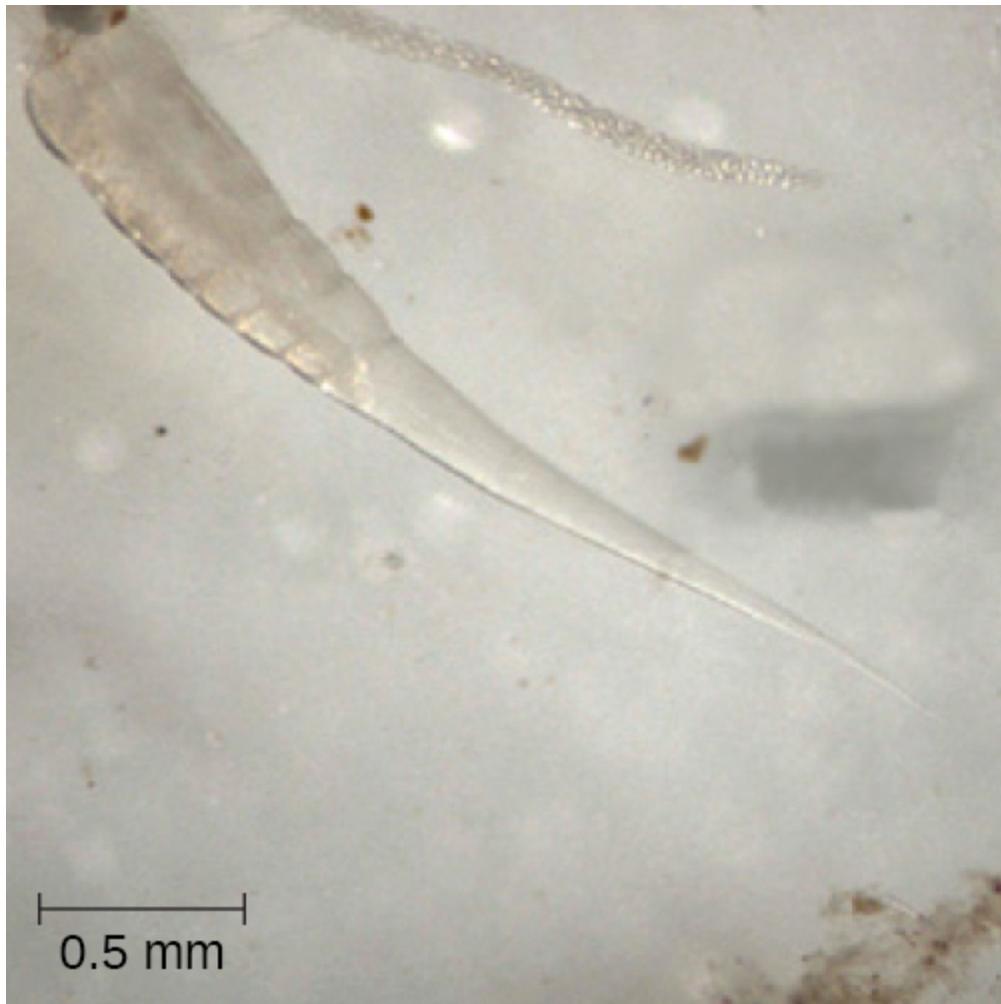
LEARNING OBJECTIVES

- Explain why we include the study of parasitic worms within the discipline of microbiology
- Compare the basic morphology of the major groups of parasitic helminthes
- Describe the characteristics of parasitic nematodes, and give an example of infective eggs and infective larvae
- Describe the characteristics of parasitic trematodes and cestodes, and give examples of each
- Identify examples of the primary causes of infections due to nematodes, trematodes, and cestodes
- Classify parasitic worms according to major groups

Parasitic helminths are animals that are often included within the study of microbiology because many species of these worms are identified by their microscopic eggs and larvae. There are two major groups of parasitic helminths: the roundworms (Nematoda) and flatworms (Platyhelminthes). Of the many species that exist in these groups, about half are parasitic and some are important human pathogens. As animals, they are multicellular and have organ systems. However, the parasitic species often have limited digestive tracts, nervous systems, and locomotor abilities. Parasitic forms may have complex reproductive cycles with several different life stages and more than one type of host. Some are **monoecious**, having both male and female reproductive organs in a single individual, while others are **dioecious**, each having either male or female reproductive organs.

Nematoda (Roundworms)

Phylum **Nematoda** (the roundworms) is a diverse group containing more than 15,000 species, of which several are important human parasites ([\[link\]](#)). These unsegmented worms have a full digestive system even when parasitic. Some are common intestinal parasites, and their eggs can sometimes be identified in feces or around the anus of infected individuals. *Ascaris lumbricoides* is the largest nematode intestinal parasite found in humans; females may reach lengths greater than 1 meter. *A. lumbricoides* is also very widespread, even in developed nations, although it is now a relatively uncommon problem in the United States. It may cause symptoms ranging from relatively mild (such as a cough and mild abdominal pain) to severe (such as intestinal blockage and impaired growth).



A micrograph of the nematode *Enterobius vermicularis*, also known as the pinworm. (credit: modification of work by Centers for Disease Control and Prevention)

Of all nematode infections in the United States, pinworm (caused by *Enterobius vermicularis*) is the most common. Pinworm causes sleeplessness and itching around the anus, where the female worms lay their eggs during the night. *Toxocara canis* and *T. cati* are nematodes found in dogs and cats, respectively, that can be transmitted to humans, causing toxocariasis. Antibodies to these parasites have been found in approximately 13.9% of the U.S. population, suggesting that exposure is common.[\[footnote\]](#) Infection can cause larval migrans, which can result in

vision loss and eye inflammation, or fever, fatigue, coughing, and abdominal pain, depending on whether the organism infects the eye or the viscera. Another common nematode infection is hookworm, which is caused by *Necator americanus* (the New World or North American hookworm) and *Ancylostoma duodenale* (the Old World hookworm). Symptoms of hookworm infection can include abdominal pain, diarrhea, loss of appetite, weight loss, fatigue, and anemia.

Won K, Kruszon-Moran D, Schantz P, Jones J. "National seroprevalence and risk factors for zoonotic *Toxocara* spp. infection." In: Abstracts of the 56th American Society of Tropical Medicine and Hygiene; Philadelphia, Pennsylvania; 2007 Nov 4-8.

Trichinellosis, also called trichinosis, caused by *Trichinella spiralis*, is contracted by consuming undercooked meat, which releases the larvae and allows them to encyst in muscles. Infection can cause fever, muscle pains, and digestive system problems; severe infections can lead to lack of coordination, breathing and heart problems, and even death. Finally, heartworm in dogs and other animals is caused by the nematode *Dirofilaria immitis*, which is transmitted by mosquitoes. Symptoms include fatigue and cough; when left untreated, death may result.

Note:

Part 2

The physician explains to Sarah's mother that ringworm can be transferred between people through touch. "It's common in school children, because they often come in close contact with each other, but anyone can become infected," he adds. "Because you can transfer it through objects, locker rooms and public pools are also a potential source of infection. It's very common among wrestlers and athletes in other contact sports."

Looking very uncomfortable, Sarah says to her mother "I want this worm out of me."

The doctor laughs and says, "Sarah, you're in luck because ringworm is just a name; it is not an actual worm. You have nothing wriggling around under your skin."

"Then what is it?" asks Sarah.

- What type of pathogen causes ringworm?

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

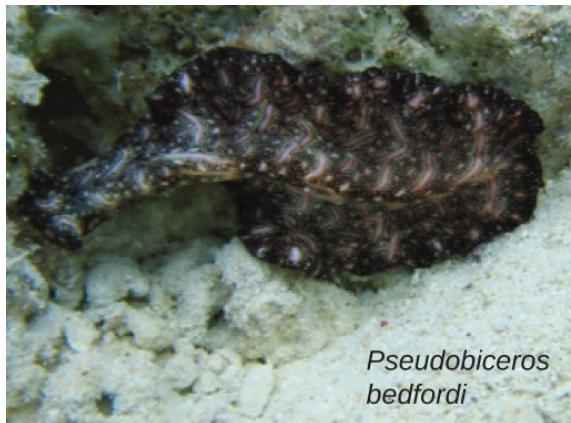
Note:

- What is the most common nematode infection in the United States?

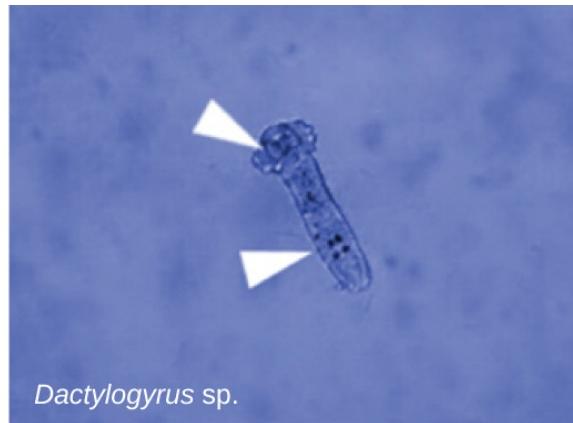
Platyhelminths (Flatworms)

Phylum **Platyhelminthes** (the platyhelminths) are flatworms. This group includes the flukes, tapeworms, and the turbellarians, which include planarians. The flukes and tapeworms are medically important parasites ([\[link\]](#)).

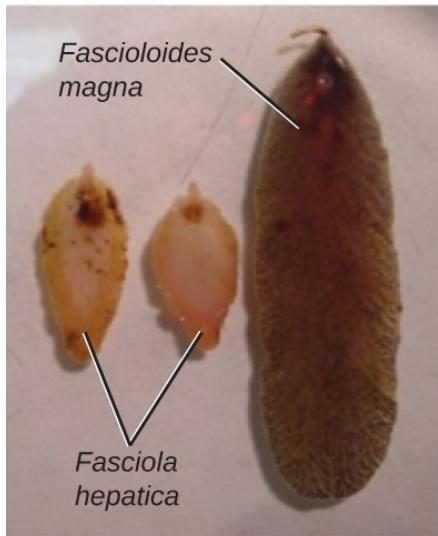
The **flukes** (trematodes) are nonsegmented flatworms that have an oral sucker ([\[link\]](#)) (and sometimes a second ventral sucker) and attach to the inner walls of intestines, lungs, large blood vessels, or the liver. Trematodes have complex life cycles, often with multiple hosts. Several important examples are the liver flukes (*Clonorchis* and *Opisthorchis*), the intestinal fluke (*Fasciolopsis buski*), and the oriental lung fluke (*Paragonimus westermani*). Schistosomiasis is a serious parasitic disease, considered second in the scale of its impact on human populations only to malaria. The parasites *Schistosoma mansoni*, *S. haematobium*, and *S. japonicum*, which are found in freshwater snails, are responsible for schistosomiasis ([\[link\]](#)). Immature forms burrow through the skin into the blood. They migrate to the lungs, then to the liver and, later, other organs. Symptoms include anemia, malnutrition, fever, abdominal pain, fluid buildup, and sometimes death.



(a) Class Turbellaria



(b) Class Monogenea



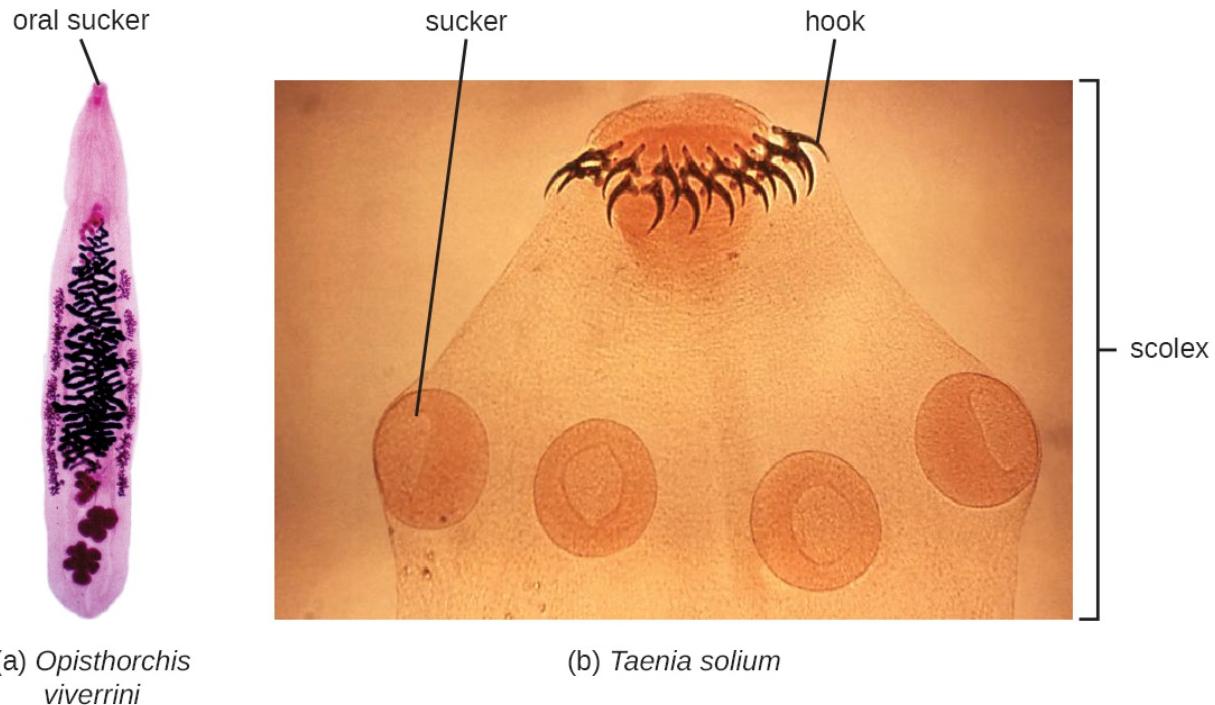
(c) Class Trematoda



(d) Class Cestoda

Phylum Platyhelminthes is divided into four classes. (a) Class Turbellaria includes the Bedford's flatworm (*Pseudobiceros bedfordi*), which is about 8–10 cm long. (b) The parasitic class Monogenea includes *Dactylogyrus* spp. Worms in this genus are commonly called gill flukes. The specimen pictured here is about 0.2 mm long and has two anchors, indicated by arrows, that it uses to latch onto the gills of host fish. (c) The Trematoda class includes the common liver fluke *Fasciola hepatica* and the giant liver fluke *Fascioloides magna* (right). The *F. magna* specimen shown here is about 7 cm long. (d) Class Cestoda includes tapeworms such as *Taenia saginata*, which infects both cattle and humans and can reach lengths of 4–10 meters; the

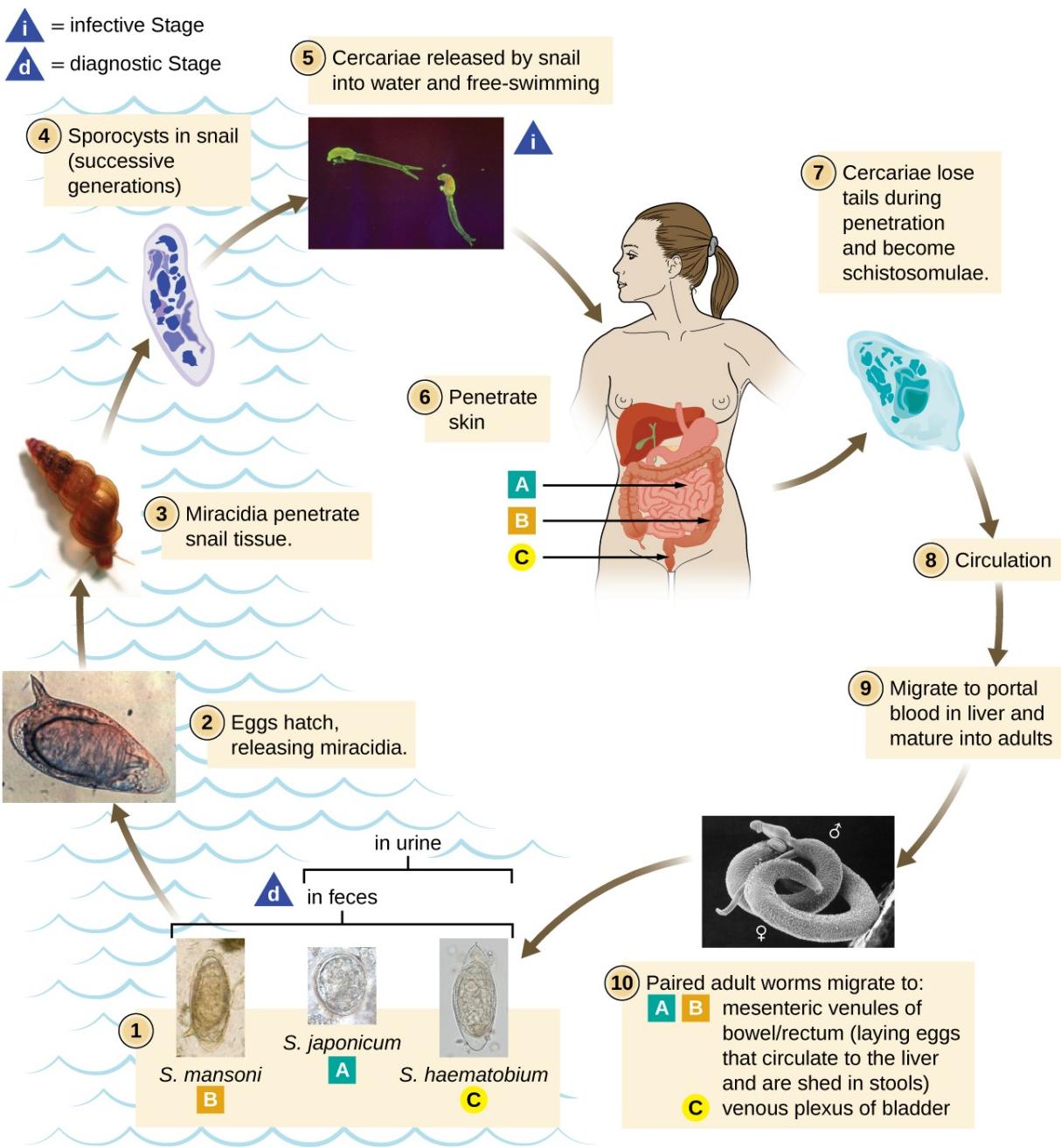
specimen shown here is about 4 meters long. (credit c: modification of work by “Flukeman”/Wikimedia Commons)



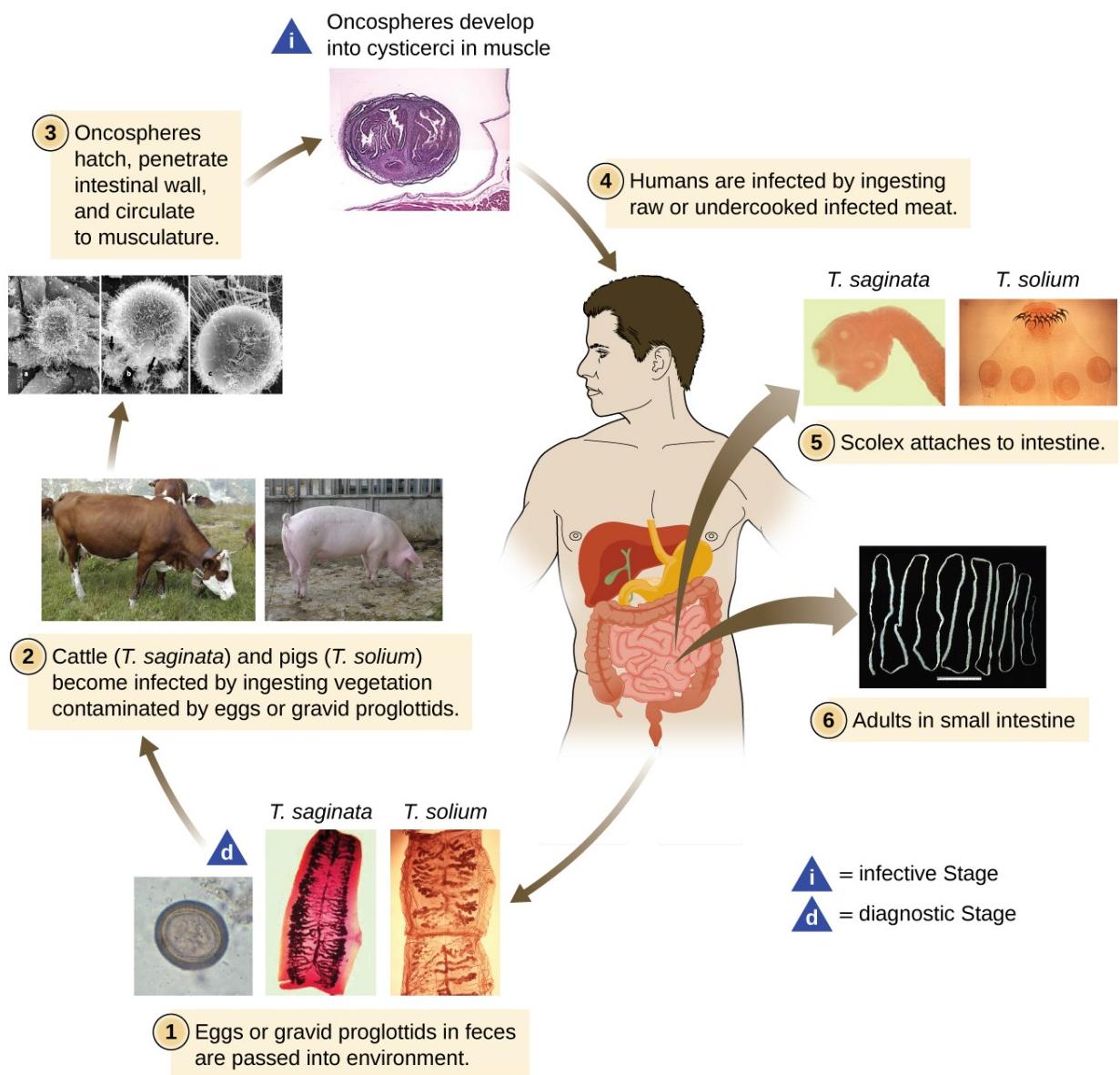
(a) The oral sucker is visible on the anterior end of this liver fluke, *Opisthorchis viverrini*. (b) This micrograph shows the scolex of the cestode *Taenia solium*, also known as the pork tapeworm. The visible suckers and hooks allow the worm to attach itself to the inner wall of the intestine. (credit a: modification of work by Sripa B, Kaewkes S, Sithithaworn P, Mairiang E, Laha T, and Smout M; credit b: modification of work by Centers for Disease Control and Prevention)

The other medically important group of platyhelminths are commonly known as **tapeworms** (cestodes) and are segmented flatworms that may have suckers or hooks at the **scolex** (head region) ([\[link\]](#)). Tapeworms use these suckers or hooks to attach to the wall of the small intestine. The body

of the worm is made up of segments called **proglottids** that contain reproductive structures; these detach when the gametes are fertilized, releasing gravid proglottids with eggs. Tapeworms often have an intermediate host that consumes the eggs, which then hatch into a larval form called an oncosphere. The oncosphere migrates to a particular tissue or organ in the intermediate host, where it forms cysticerci. After being eaten by the definitive host, the cysticerci develop into adult tapeworms in the host's digestive system ([\[link\]](#)). *Taenia saginata* (the beef tapeworm) and *T. solium* (the pork tapeworm) enter humans through ingestion of undercooked, contaminated meat. The adult worms develop and reside in the intestine, but the larval stage may migrate and be found in other body locations such as skeletal and smooth muscle. The beef tapeworm is relatively benign, although it can cause digestive problems and, occasionally, allergic reactions. The pork tapeworm can cause more serious problems when the larvae leave the intestine and colonize other tissues, including those of the central nervous system. *Diphyllobothrium latum* is the largest human tapeworm and can be ingested in undercooked fish. It can grow to a length of 15 meters. *Echinococcus granulosus*, the dog tapeworm, can parasitize humans and uses dogs as an important host.



The life cycle of *Schistosoma* spp. includes several species of water snails, which serve as secondary hosts. The parasite is transmitted to humans through contact with contaminated water and takes up residence in the veins of the digestive system. Eggs escape the host in the urine or feces and infect a snail to complete the life cycle. (credit “illustration”: modification of work by Centers for Disease Control and Prevention; credit “step 3 photo”: modification of work by Fred A. Lewis, Yung-san Liang, Nithya Raghavan & Matty Knight)



Life cycle of a tapeworm. (credit “illustration”: modification of work by Centers for Disease Control and Prevention; credit “step 3 micrographs”: modification of work by American Society for Microbiology)

Note:

- What group of medically important flatworms is segmented and what group is unsegmented?

Note:**Food for Worms?**

For residents of temperate, developed countries, it may be difficult to imagine just how common helminth infections are in the human population. In fact, they are quite common and even occur frequently in the United States. Worldwide, approximately 807–1,221 million people are infected with *Ascaris lumbricoides* (perhaps one-sixth of the human population) and far more are infected if all nematode species are considered.[\[footnote\]](#) Rates of infection are relatively high even in industrialized nations. Approximately 604–795 million people are infected with whipworm (*Trichuris*) worldwide (*Trichuris* can also infect dogs), and 576–740 million people are infected with hookworm (*Necator americanus* and *Ancylostoma duodenale*).[\[footnote\]](#) *Toxocara*, a nematode parasite of dogs and cats, is also able to infect humans. It is widespread in the United States, with about 10,000 symptomatic cases annually. However, one study found 14% of the population (more than 40 million Americans) was seropositive, meaning they had been exposed to the parasite at one time. More than 200 million people have schistosomiasis worldwide. Most of the World Health Organization (WHO) neglected tropical diseases are helminths. In some cases, helminths may cause subclinical illnesses, meaning the symptoms are so mild that they go unnoticed. In other cases, the effects may be more severe or chronic, leading to fluid accumulation and organ damage. With so many people affected, these parasites constitute a major global public health concern.

Fenwick, A. “The global burden of neglected tropical diseases.” *Public health* 126 no.3 (Mar 2012): 233–6.

de Silva, N., et. al. (2003). “Soil-transmitted helminth infections: updating the global picture”. *Trends in Parasitology* 19 (December 2003): 547–51.

Note:

Eradicating the Guinea Worm

Dracunculiasis, or Guinea worm disease, is caused by a nematode called *Dracunculus medinensis*. When people consume contaminated water, water fleas (small crustaceans) containing the nematode larvae may be ingested. These larvae migrate out of the intestine, mate, and move through the body until females eventually emerge (generally through the feet).

While Guinea worm disease is rarely fatal, it is extremely painful and can be accompanied by secondary infections and edema ([\[link\]](#)).



The Guinea worm can be removed from a leg vein of an infected person by gradually winding it around a stick, like this matchstick. (credit: Centers for Disease Control and Prevention)

An eradication campaign led by WHO, the CDC, the United Nations Children's Fund (UNICEF), and the Carter Center (founded by former U.S. president Jimmy Carter) has been extremely successful in reducing cases of dracunculiasis. This has been possible because diagnosis is straightforward, there is an inexpensive method of control, there is no animal reservoir, the water fleas are not airborne (they are restricted to still

water), the disease is geographically limited, and there has been a commitment from the governments involved. Additionally, no vaccines or medication are required for treatment and prevention. In 1986, 3.5 million people were estimated to be affected. After the eradication campaign, which included helping people in affected areas learn to filter water with cloth, only four countries continue to report the disease (Chad, Mali, South Sudan, and Ethiopia) with a total of 126 cases reported to WHO in 2014.

[footnote]

World Health Organization. “South Sudan Reports Zero Cases of Guinea-Worm Disease for Seventh Consecutive Month.” 2016.

http://www.who.int/dracunculiasis/no_new_case_for_seventh_consecutive_months/en/. Accessed May 2, 2016.

Key Concepts and Summary

- Helminth parasites are included within the study of microbiology because they are often identified by looking for microscopic eggs and larvae.
- The two major groups of helminth parasites are the roundworms (Nematoda) and the flatworms (Platyhelminthes).
- Nematodes are common intestinal parasites often transmitted through undercooked foods, although they are also found in other environments.
- Platyhelminths include **tapeworms** and **flukes**, which are often transmitted through undercooked meat.

Multiple Choice

Exercise:

Problem: A fluke is classified within which of the following?

- A. Nematoda
- B. Rotifera
- C. Platyhelminthes

D. Annelida

Solution:

C

Exercise:

Problem:

A nonsegmented worm is found during a routine colonoscopy of an individual who reported having abdominal cramps, nausea, and vomiting. This worm is likely which of the following?

- A. nematode
 - B. fluke
 - C. trematode
 - D. annelid
-

Solution:

A

Exercise:

Problem:

A segmented worm has male and female reproductive organs in each segment. Some use hooks to attach to the intestinal wall. Which type of worm is this?

- A. fluke
 - B. nematode
 - C. cestode
 - D. annelid
-

Solution:

C

Fill in the Blank

Exercise:

Problem: Flukes are in class _____.

Solution:

Trematoda

Exercise:

Problem:

A species of worm in which there are distinct male and female individuals is described as _____.

Solution:

dioecious

Short Answer

Exercise:

Problem: What is the best defense against tapeworm infection?

Critical Thinking

Exercise:

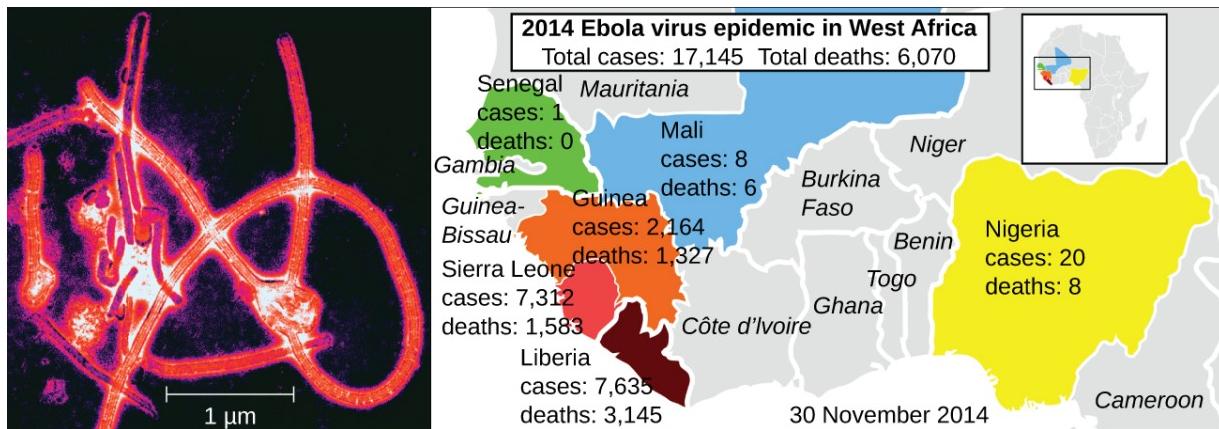
Problem:

Given the life cycle of the *Schistosoma* parasite, suggest a method of prevention of the disease.

Virus, Viroids, and Prions - Introduction

class="introduction"

The year
2014 saw
the first
large-scale
outbreak of
Ebola virus
(electron
micrograph,
left) in
human
populations
in West
Africa
(right). Such
epidemics
are now
widely
reported and
documented
, but viral
epidemics
are sure to
have
plagued
human
populations
since the
origin of our
species.
(credit left:
modificatio
n of work
by Thomas
W. Geisbert)



Public health measures in the developed world have dramatically reduced mortality from viral epidemics. But when epidemics do occur, they can spread quickly with global air travel. In 2009, an outbreak of H1N1 influenza spread across various continents. In early 2014, cases of Ebola in Guinea led to a massive epidemic in western Africa. This included the case of an infected man who traveled to the United States, sparking fears the epidemic might spread beyond Africa.

Until the late 1930s and the advent of the electron microscope, no one had seen a virus. Yet treatments for preventing or curing viral infections were used and developed long before that. Historical records suggest that by the 17th century, and perhaps earlier, inoculation (also known as variolation) was being used to prevent the viral disease smallpox in various parts of the world. By the late 18th century, Englishman Edward Jenner was inoculating patients with cowpox to prevent smallpox, a technique he coined *vaccination*.[\[footnote\]](#)

S. Riedel “Edward Jenner and the History of Smallpox and Vaccination.” *Baylor University Medical Center Proceedings* 18, no. 1 (January 2005): 21–25.

Today, the structure and genetics of viruses are well defined, yet new discoveries continue to reveal their complexities. In this chapter, we will learn about the structure, classification, and cultivation of viruses, and how they impact their hosts. In addition, we will learn about other infective particles such as viroids and prions.

LEARNING OBJECTIVES

- Describe the general characteristics of viruses as pathogens
- Describe viral genomes
- Describe the general characteristics of viral life cycles
- Differentiate among bacteriophages, plant viruses, and animal viruses
- Describe the characteristics used to identify viruses as obligate intracellular parasites

Despite their small size, which prevented them from being seen with light microscopes, the discovery of a filterable component smaller than a bacterium that causes tobacco mosaic disease (TMD) dates back to 1892.[\[footnote\]](#) At that time, Dmitri Ivanovski, a Russian botanist, discovered the source of TMD by using a porcelain filtering device first invented by Charles Chamberland and Louis Pasteur in Paris in 1884. Porcelain Chamberland filters have a pore size of 0.1 μm , which is small enough to remove all bacteria $\geq 0.2 \mu\text{m}$ from any liquids passed through the device. An extract obtained from TMD-infected tobacco plants was made to determine the cause of the disease. Initially, the source of the disease was thought to be bacterial. It was surprising to everyone when Ivanovski, using a Chamberland filter, found that the cause of TMD was not removed after passing the extract through the porcelain filter. So if a bacterium was not the cause of TMD, what could be causing the disease? Ivanovski concluded the cause of TMD must be an extremely small bacterium or bacterial spore. Other scientists, including Martinus Beijerinck, continued investigating the cause of TMD. It was Beijerinck, in 1899, who eventually concluded the causative agent was not a bacterium but, instead, possibly a chemical, like a biological poison we would describe today as a toxin. As a result, the word *virus*, Latin for poison, was used to describe the cause of TMD a few years after Ivanovski's initial discovery. Even though he was not able to see the virus that caused TMD, and did not realize the cause was not a bacterium, Ivanovski is credited as the original discoverer of viruses and a founder of the field of virology.

H. Lecoq. “[Discovery of the First Virus, the Tobacco Mosaic Virus: 1892 or 1898?].” *Comptes Rendus de l’Academie des Sciences – Serie III – Sciences de la Vie* 324, no. 10 (2001): 929–933.

Today, we can see viruses using electron microscopes ([\[link\]](#)) and we know much more about them. Viruses are distinct biological entities; however, their evolutionary origin is still a matter of speculation. In terms of taxonomy, they are not included in the tree of life because they are **acellular** (not consisting of cells). In order to survive and reproduce, viruses must infect a cellular host, making them obligate intracellular parasites. The genome of a virus enters a host cell and directs the production of the viral components, proteins and nucleic acids, needed to form new virus particles called **virions**. New virions are made in the host cell by assembly of viral components. The new virions transport the viral genome to another host cell to carry out another round of infection. [\[link\]](#) summarizes the properties of viruses.

Characteristics of Viruses

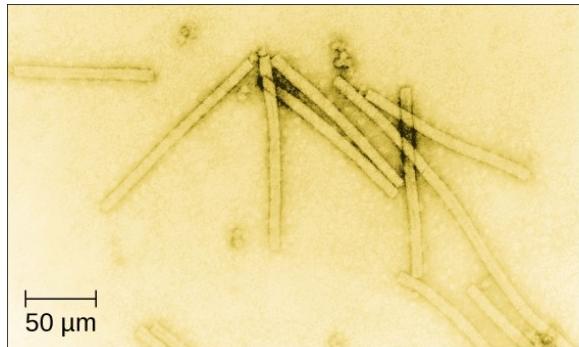
Infectious, acellular pathogens

Obligate intracellular parasites with host and cell-type specificity

DNA or RNA genome (never both)

Genome is surrounded by a protein capsid and, in some cases, a phospholipid membrane studded with viral glycoproteins

Lack genes for many products needed for successful reproduction, requiring exploitation of host-cell genomes to reproduce



(a)



(b)

(a) Tobacco mosaic virus (TMV) viewed with transmission electron microscope. (b) Plants infected with tobacco mosaic disease (TMD), caused by TMV. (credit a: modification of work by USDA Agricultural Research Service—scale-bar data from Matt Russell; credit b: modification of work by USDA Forest Service, Department of Plant Pathology Archive North Carolina State University)

Note:

- Why was the first virus investigated mistaken for a toxin?

Hosts and Viral Transmission

Viruses can infect every type of host cell, including those of plants, animals, fungi, protists, bacteria, and archaea. Most viruses will only be able to infect the cells of one or a few species of organism. This is called the **host range**. However, having a wide host range is not common and viruses will typically only infect specific hosts and only specific cell types within those hosts. The viruses that infect bacteria are called **bacteriophages**, or simply phages. The word *phage* comes from the Greek word for devour. Other viruses are just identified by their host group, such as animal or plant viruses. Once a cell is infected, the effects of the virus can vary depending on the type of virus. Viruses may cause abnormal growth of the cell or cell death, alter the cell's genome, or cause little noticeable effect in the cell.

Viruses can be transmitted through direct contact, indirect contact with fomites, or through a **vector**: an animal that transmits a pathogen from one host to another.

Arthropods such as mosquitoes, ticks, and flies, are typical vectors for viral diseases, and they may act as **mechanical vectors** or **biological vectors**.

Mechanical transmission occurs when the arthropod carries a viral pathogen on the outside of its body and transmits it to a new host by physical contact. Biological transmission occurs when the arthropod carries the viral pathogen inside its body and transmits it to the new host through biting.

In humans, a wide variety of viruses are capable of causing various infections and diseases. Some of the deadliest emerging pathogens in humans are viruses, yet we have few treatments or drugs to deal with viral infections, making them difficult to eradicate.

Viruses that can be transmitted from an animal host to a human host can cause zoonoses. For example, the avian influenza virus originates in birds, but can cause disease in humans. Reverse zoonoses are caused by infection of an animal by a virus that originated in a human.

Note:

Fighting Bacteria with Viruses

The emergence of superbugs, or multidrug resistant bacteria, has become a major challenge for pharmaceutical companies and a serious health-care problem.

According to a 2013 report by the US Centers for Disease Control and Prevention (CDC), more than 2 million people are infected with drug-resistant bacteria in the US annually, resulting in at least 23,000 deaths.[\[footnote\]](#) The continued use and overuse of antibiotics will likely lead to the evolution of even more drug-resistant strains.

US Department of Health and Human Services, Centers for Disease Control and Prevention. "Antibiotic Resistance Threats in the United States, 2013."

<http://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf> (accessed September 22, 2015).

One potential solution is the use of phage therapy, a procedure that uses bacteria-killing viruses (bacteriophages) to treat bacterial infections. Phage therapy is not a new idea. The discovery of bacteriophages dates back to the early 20th century, and phage therapy was first used in Europe in 1915 by the English bacteriologist Frederick Twort.[\[footnote\]](#) However, the subsequent discovery of penicillin and other antibiotics led to the near abandonment of this form of therapy, except in the former Soviet Union and a few countries in Eastern Europe. Interest in phage

therapy outside of the countries of the former Soviet Union is only recently re-emerging because of the rise in antibiotic-resistant bacteria.[\[footnote\]](#)

M. Clokie et al. "Phages in Nature." *Bacteriophage* 1, no. 1 (2011): 31–45.

A. Sulakvelidze et al. "Bacteriophage Therapy." *Antimicrobial Agents and Chemotherapy* 45, no. 3 (2001): 649–659.

Phage therapy has some advantages over antibiotics in that phages kill only one specific bacterium, whereas antibiotics kill not only the pathogen but also beneficial bacteria of the normal microbiota. Development of new antibiotics is also expensive for drug companies and for patients, especially for those who live in countries with high poverty rates.

Phages have also been used to prevent food spoilage. In 2006, the US Food and Drug Administration approved the use of a solution containing six bacteriophages that can be sprayed on lunch meats such as bologna, ham, and turkey to kill *Listeria monocytogenes*, a bacterium responsible for listeriosis, a form of food poisoning. Some consumers have concerns about the use of phages on foods, however, especially given the rising popularity of organic products. Foods that have been treated with phages must declare "bacteriophage preparation" in the list of ingredients or include a label declaring that the meat has been "treated with antimicrobial solution to reduce microorganisms."[\[footnote\]](#)

US Food and Drug Administration. "FDA Approval of *Listeria*-specific Bacteriophage Preparation on Ready-to-Eat (RTE) Meat and Poultry Products." <http://www.fda.gov/food/ingredientspackaginglabeling/ucm083572.htm> (accessed September 22, 2015).

Note:

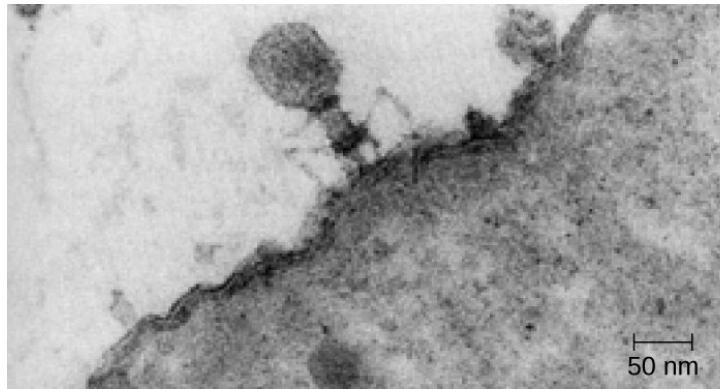
- Why do humans not have to be concerned about the presence of bacteriophages in their food?
- What are three ways that viruses can be transmitted between hosts?

Viral Structures

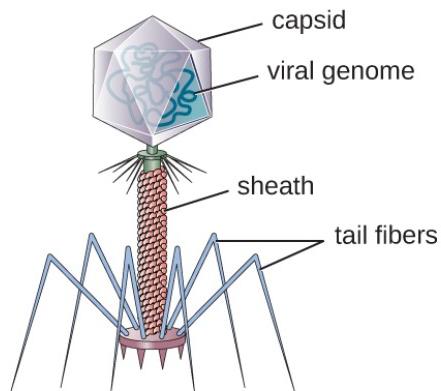
In general, virions (viral particles) are small and cannot be observed using a regular light microscope. They are much smaller than prokaryotic and eukaryotic cells; this is an adaptation allowing viruses to infect these larger cells (see [\[link\]](#)). The size of a virion can range from 20 nm for small viruses up to 900 nm for typical, large

viruses (see [\[link\]](#)). Recent discoveries, however, have identified new giant viral species, such as *Pandoravirus salinus* and *Pithovirus sibericum*, with sizes approaching that of a bacterial cell.[\[footnote\]](#)

N. Philippe et al. “Pandoraviruses: Amoeba Viruses with Genomes up to 2.5 Mb Reaching that of Parasitic Eukaryotes.” *Science* 341, no. 6143 (2013): 281–286.

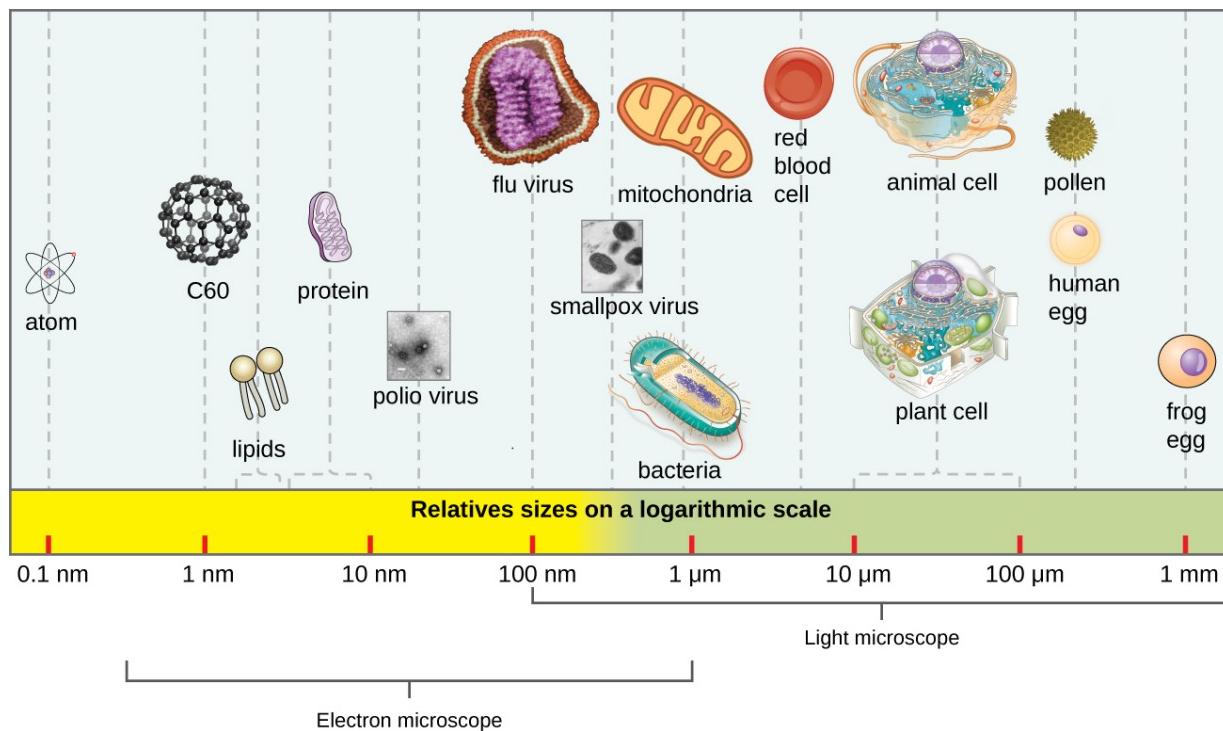


(a)



(b)

(a) In this transmission electron micrograph, a bacteriophage (a virus that infects bacteria) is dwarfed by the bacterial cell it infects. (b) An illustration of the bacteriophage in the micrograph. (credit a: modification of work by U.S. Department of Energy, Office of Science, LBL, PBD)



The size of a virus is small relative to the size of most bacterial and eukaryotic cells and their organelles.

In 1935, after the development of the electron microscope, Wendell Stanley was the first scientist to crystallize the structure of the tobacco mosaic virus and discovered that it is composed of RNA and protein. In 1943, he isolated *Influenza B virus*, which contributed to the development of an influenza (flu) vaccine. Stanley's discoveries unlocked the mystery of the nature of viruses that had been puzzling scientists for over 40 years and his contributions to the field of virology led to him being awarded the Nobel Prize in 1946.

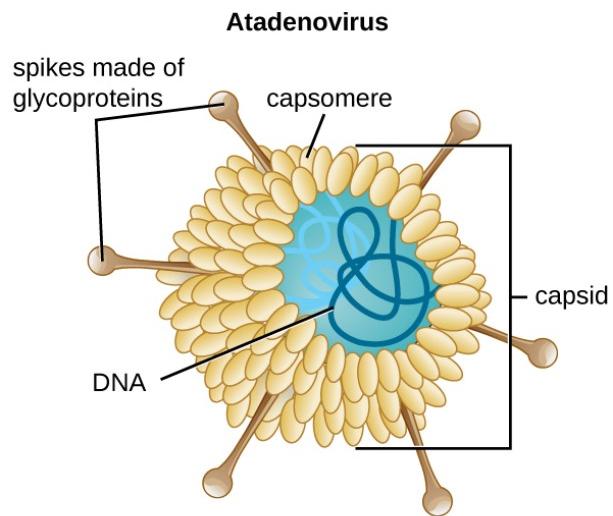
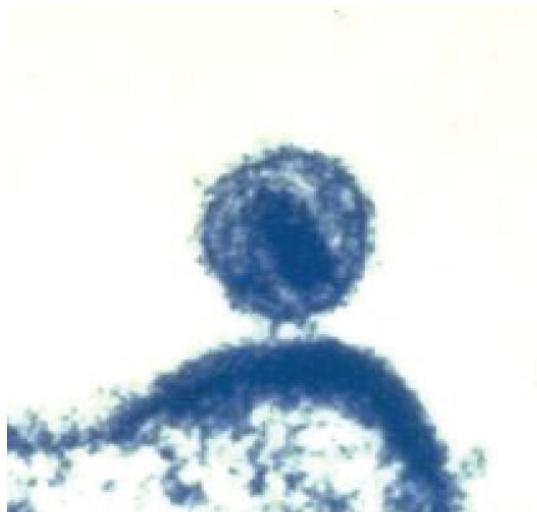
As a result of continuing research into the nature of viruses, we now know they consist of a nucleic acid (either RNA or DNA, but never both) surrounded by a protein coat called a **capsid** (see [\[link\]](#)). The interior of the capsid is not filled with cytosol, as in a cell, but instead it contains the bare necessities in terms of genome and enzymes needed to direct the synthesis of new virions. Each capsid is composed of protein subunits called **capsomeres** made of one or more different types of capsomere proteins that interlock to form the closely packed capsid.

There are two categories of viruses based on general composition. Viruses formed from only a nucleic acid and capsid are called **naked viruses** or **nonenveloped**

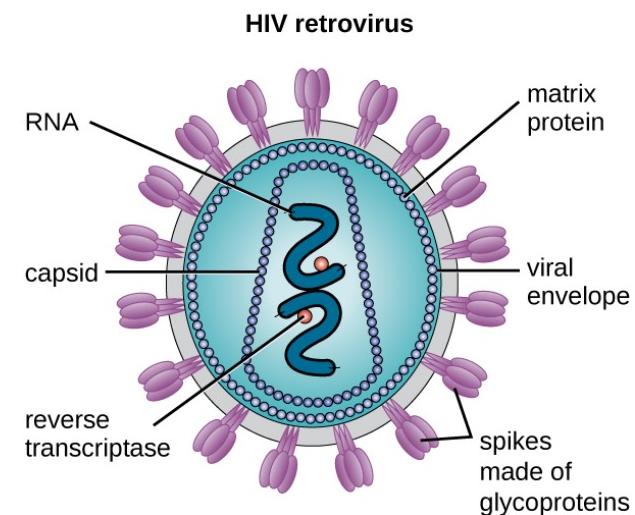
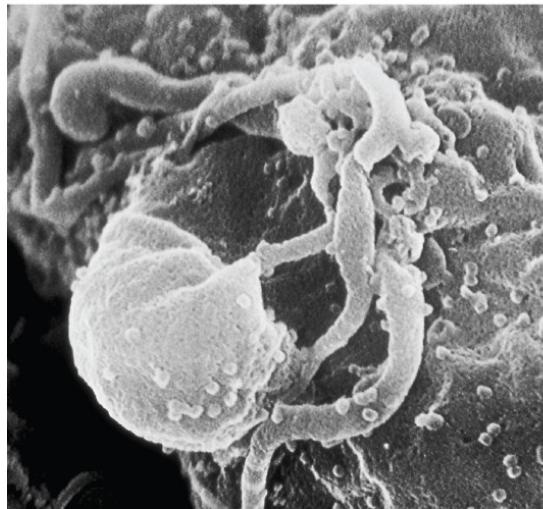
viruses. Viruses formed with a nucleic-acid packed capsid surrounded by a lipid layer are called **enveloped viruses** (see [[link](#)]). The **viral envelope** is a small portion of phospholipid membrane obtained as the virion buds from a host cell. The viral envelope may either be intracellular or cytoplasmic in origin.

Extending outward and away from the capsid on some naked viruses and enveloped viruses are protein structures called **spikes**. At the tips of these spikes are structures that allow the virus to attach and enter a cell, like the influenza virus hemagglutinin spikes (H) or enzymes like the neuraminidase (N) influenza virus spikes that allow the virus to detach from the cell surface during release of new virions. Influenza viruses are often identified by their H and N spikes. For example, H1N1 influenza viruses were responsible for the pandemics in 1918 and 2009,[[footnote](#)] H2N2 for the pandemic in 1957, and H3N2 for the pandemic in 1968.

J. Cohen. “What’s Old Is New: 1918 Virus Matches 2009 H1N1 Strain. *Science* 327, no. 5973 (2010): 1563–1564.



(a)

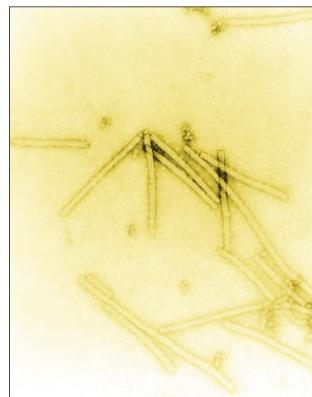


(b)

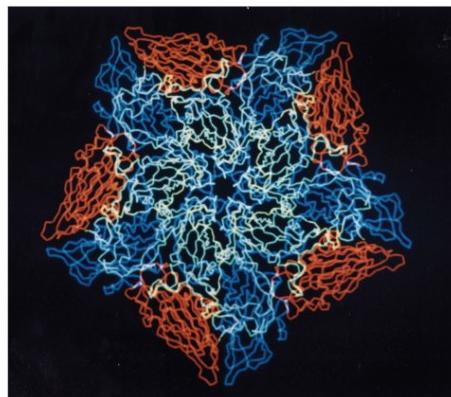
(a) The naked atadenovirus uses spikes made of glycoproteins from its capsid to bind to host cells. (b) The enveloped human immunodeficiency virus uses spikes made of glycoproteins embedded in its envelope to bind to host cells
 (credit a “micrograph”: modification of work by NIAID; credit b “micrograph”: modification of work by Centers for Disease Control and Prevention)

Viruses vary in the shape of their capsids, which can be either **helical**, **polyhedral**, or **complex**. A helical capsid forms the shape of tobacco mosaic virus (TMV), a

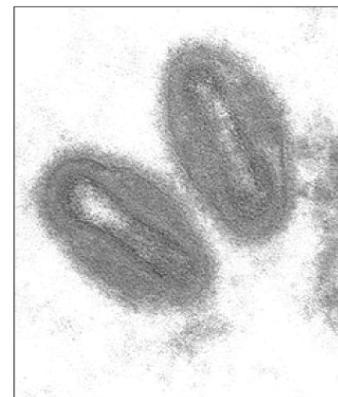
naked helical virus, and Ebola virus, an enveloped helical virus. The capsid is cylindrical or rod shaped, with the genome fitting just inside the length of the capsid. Polyhedral capsids form the shapes of poliovirus and rhinovirus, and consist of a nucleic acid surrounded by a polyhedral (many-sided) capsid in the form of an icosahedron. An **icosahedral** capsid is a three-dimensional, 20-sided structure with 12 vertices. These capsids somewhat resemble a soccer ball. Both helical and polyhedral viruses can have envelopes. Viral shapes seen in certain types of bacteriophages, such as T4 phage, and poxviruses, like vaccinia virus, may have features of both polyhedral and helical viruses so they are described as a complex viral shape (see [\[link\]](#)). In the bacteriophage complex form, the genome is located within the polyhedral head and the **sheath** connects the head to the **tail fibers** and **tail pins** that help the virus attach to receptors on the host cell's surface. Poxviruses that have complex shapes are often brick shaped, with intricate surface characteristics not seen in the other categories of capsid.



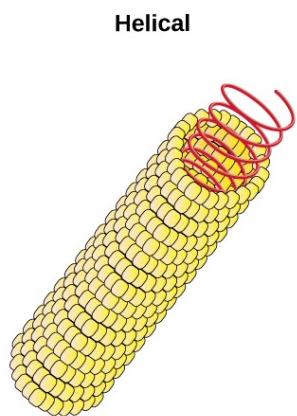
Tobacco mosaic virus



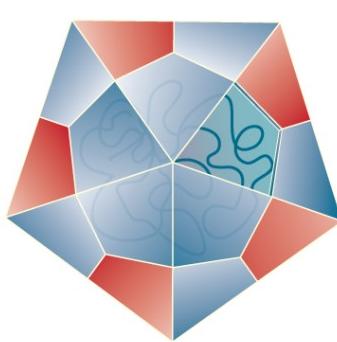
Human rhinovirus HRV14



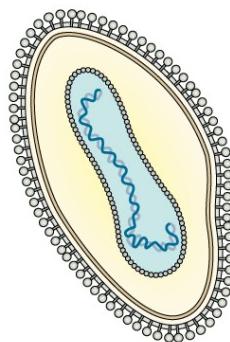
Variola virus



(a)



(b)



(c)

Viral capsids can be (a) helical, (b) polyhedral, or (c) have a complex shape.

(credit a “micrograph”: modification of work by USDA ARS; credit b “micrograph”: modification of work by U.S. Department of Energy)

Note:

- Which types of viruses have spikes?

Classification and Taxonomy of Viruses

Although viruses are not classified in the three domains of life, their numbers are great enough to require classification. Since 1971, the International Union of Microbiological Societies Virology Division has given the task of developing, refining, and maintaining a universal virus taxonomy to the International Committee on Taxonomy of Viruses (ICTV). Since viruses can mutate so quickly, it can be difficult to classify them into a genus and a species epithet using the binomial nomenclature system. Thus, the ICTV’s viral nomenclature system classifies viruses into families and genera based on viral genetics, chemistry, morphology, and mechanism of multiplication. To date, the ICTV has classified known viruses in seven orders, 96 families, and 350 genera. Viral family names end in *-viridae* (e.g., *Parvoviridae*) and genus names end in *-virus* (e.g., *Parvovirus*). The names of viral orders, families, and genera are all italicized. When referring to a viral species, we often use a genus and species epithet such as *Pandoravirus dulcis* or *Pandoravirus salinus*.

The Baltimore classification system is an alternative to ICTV nomenclature. The Baltimore system classifies viruses according to their genomes (DNA or RNA, single versus double stranded, and mode of replication). This system thus creates seven groups of viruses that have common genetics and biology.

Note:



Explore the latest virus [taxonomy](#) at the ICTV website.

Aside from formal systems of nomenclature, viruses are often informally grouped into categories based on chemistry, morphology, or other characteristics they share in common. Categories may include naked or enveloped structure, single-stranded (ss) or double-stranded (ds) DNA or ss or ds RNA genomes, segmented or nonsegmented genomes, and positive-strand (+) or negative-strand (-) RNA. For example, herpes viruses can be classified as a dsDNA enveloped virus; human immunodeficiency virus (HIV) is a +ssRNA enveloped virus, and tobacco mosaic virus is a +ssRNA virus. Other characteristics such as host specificity, tissue specificity, capsid shape, and special genes or enzymes may also be used to describe groups of similar viruses. [\[link\]](#) lists some of the most common viruses that are human pathogens by genome type.

Common Pathogenic Viruses

Genome	Family	Example Virus	Clinical Features
dsDNA, enveloped	<i>Poxviridae</i>	<i>Orthopoxvirus</i>	Skin papules, pustules, lesions
	<i>Poxviridae</i>	<i>Parapoxvirus</i>	Skin lesions

Common Pathogenic Viruses			
Genome	Family	Example Virus	Clinical Features
dsDNA, naked	<i>Herpesviridae</i>	<i>Simplexvirus</i>	Cold sores, genital herpes, sexually transmitted disease
	<i>Adenoviridae</i>	<i>Adenovirus</i>	Respiratory infection (common cold)
	<i>Papillomaviridae</i>	<i>Papillomavirus</i>	Genital warts, cervical, vulvar, or vaginal cancer
	<i>Reoviridae</i>	<i>Reovirus</i>	Gastroenteritis severe diarrhea (stomach flu)
ssDNA, naked	<i>Parvoviridae</i>	<i>Adeno-associated dependoparvovirus A</i>	Respiratory tract infection
	<i>Parvoviridae</i>	<i>Adeno-associated dependoparvovirus B</i>	Respiratory tract infection
dsRNA, naked	<i>Reoviridae</i>	<i>Rotavirus</i>	Gastroenteritis
+ssRNA, naked	<i>Picornaviridae</i>	<i>Enterovirus C</i>	Poliomyelitis

Common Pathogenic Viruses			
Genome	Family	Example Virus	Clinical Features
+ssRNA, enveloped	<i>Picornaviridae</i>	<i>Rhinovirus</i>	Upper respiratory tract infection (common cold)
	<i>Picornaviridae</i>	<i>Hepadovirus</i>	Hepatitis
	<i>Togaviridae</i>	<i>Alphavirus</i>	Encephalitis, hemorrhagic fever
	<i>Togaviridae</i>	<i>Rubivirus</i>	Rubella
	<i>Retroviridae</i>	<i>Lentivirus</i>	Acquired immune deficiency syndrome (AIDS)
	<i>Filoviridae</i>	<i>Zaire Ebolavirus</i>	Hemorrhagic fever
-ssRNA, enveloped	<i>Orthomyxoviridae</i>	<i>Influenzavirus A, B, C</i>	Flu
	<i>Rhabdoviridae</i>	<i>Lyssavirus</i>	Rabies

Note:

- What are the types of virus genomes?

Classification of Viral Diseases

While the ICTV has been tasked with the biological classification of viruses, it has also played an important role in the classification of diseases caused by viruses. To facilitate the tracking of virus-related human diseases, the ICTV has created classifications that link to the International Classification of Diseases (ICD), the standard taxonomy of disease that is maintained and updated by the World Health Organization (WHO). The ICD assigns an alphanumeric code of up to six characters to every type of viral infection, as well as all other types of diseases, medical conditions, and causes of death. This ICD code is used in conjunction with two other coding systems (the Current Procedural Terminology, and the Healthcare Common Procedure Coding System) to categorize patient conditions for treatment and insurance reimbursement.

For example, when a patient seeks treatment for a viral infection, ICD codes are routinely used by clinicians to order laboratory tests and prescribe treatments specific to the virus suspected of causing the illness. This ICD code is then used by medical laboratories to identify tests that must be performed to confirm the diagnosis. The ICD code is used by the health-care management system to verify that all treatments and laboratory work performed are appropriate for the given virus. Medical coders use ICD codes to assign the proper code for procedures performed, and medical billers, in turn, use this information to process claims for reimbursement by insurance companies. Vital-records keepers use ICD codes to record cause of death on death certificates, and epidemiologists use ICD codes to calculate morbidity and mortality statistics.

Key Concepts and Summary

- Viruses are generally ultramicroscopic, typically from 20 nm to 900 nm in length. Some large viruses have been found.
- **Virions** are acellular and consist of a nucleic acid, DNA or RNA, but not both, surrounded by a protein **capsid**. There may also be a phospholipid membrane surrounding the capsid.
- Viruses are obligate intracellular parasites.
- Viruses are known to infect various types of cells found in plants, animals, fungi, protists, bacteria, and archaea. Viruses typically have limited **host ranges** and infect specific cell types.
- Viruses may have **helical, polyhedral, or complex** shapes.
- Classification of viruses is based on morphology, type of nucleic acid, host range, cell specificity, and enzymes carried within the virion.
- Like other diseases, viral diseases are classified using ICD codes.

Short Answer

Exercise:

Problem:

Discuss the geometric differences among helical, polyhedral, and complex viruses.

Exercise:

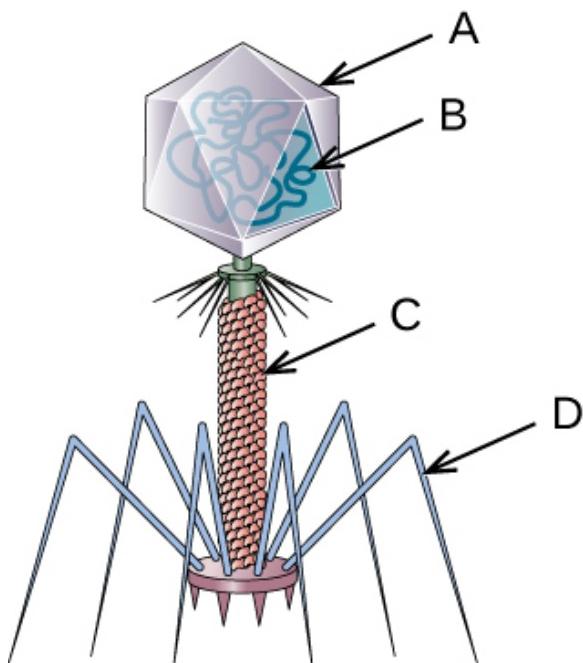
Problem:

What was the meaning of the word “virus” in the 1880s and why was it used to describe the cause of tobacco mosaic disease?

Critical Thinking

Exercise:

Problem: Name each labeled part of the illustrated bacteriophage.



Exercise:

Problem:

In terms of evolution, which do you think arises first? The virus or the host?
Explain your answer.

Exercise:

Problem:

Do you think it is possible to create a virus in the lab? Imagine that you are a mad scientist. Describe how you would go about creating a new virus.

The Viral Life Cycle

LEARNING OBJECTIVES

- Describe the lytic and lysogenic life cycles
- Describe the replication process of animal viruses
- Describe unique characteristics of retroviruses and latent viruses
- Discuss human viruses and their virus-host cell interactions
- Explain the process of transduction
- Describe the replication process of plant viruses

All viruses depend on cells for reproduction and metabolic processes. By themselves, viruses do not encode for all of the enzymes necessary for viral replication. But within a host cell, a virus can commandeer cellular machinery to produce more viral particles. Bacteriophages replicate only in the cytoplasm, since prokaryotic cells do not have a nucleus or organelles. In eukaryotic cells, most DNA viruses can replicate inside the nucleus, with an exception observed in the large DNA viruses, such as the poxviruses, that can replicate in the cytoplasm. RNA viruses that infect animal cells often replicate in the cytoplasm.

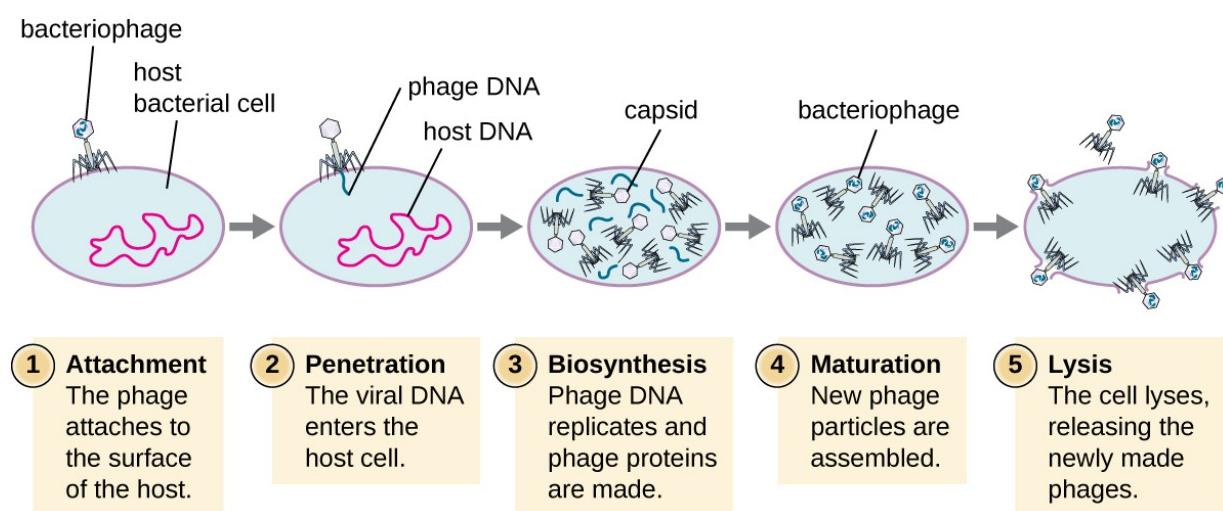
The Life Cycle of Viruses with Prokaryote Hosts

The life cycle of bacteriophages has been a good model for understanding how viruses affect the cells they infect, since similar processes have been observed for eukaryotic viruses, which can cause immediate death of the cell or establish a latent or chronic infection. **Virulent phages** typically lead to the death of the cell through cell lysis. **Temperate phages**, on the other

hand, can become part of a host chromosome and are replicated with the cell genome until such time as they are induced to make newly assembled viruses, or **progeny viruses**.

The Lytic Cycle

During the **lytic cycle** of virulent phage, the bacteriophage takes over the cell, reproduces new phages, and destroys the cell. T-even phage is a good example of a well-characterized class of virulent phages. There are five stages in the bacteriophage lytic cycle (see [\[link\]](#)). **Attachment** is the first stage in the infection process in which the phage interacts with specific bacterial surface receptors (e.g., lipopolysaccharides and OmpC protein on host surfaces). Most phages have a narrow host range and may infect one species of bacteria or one strain within a species. This unique recognition can be exploited for targeted treatment of bacterial infection by phage therapy or for phage typing to identify unique bacterial subspecies or strains. The second stage of infection is entry or **penetration**. This occurs through contraction of the tail sheath, which acts like a hypodermic needle to inject the viral genome through the cell wall and membrane. The phage head and remaining components remain outside the bacteria.



A virulent phage shows only the lytic cycle pictured here. In the lytic

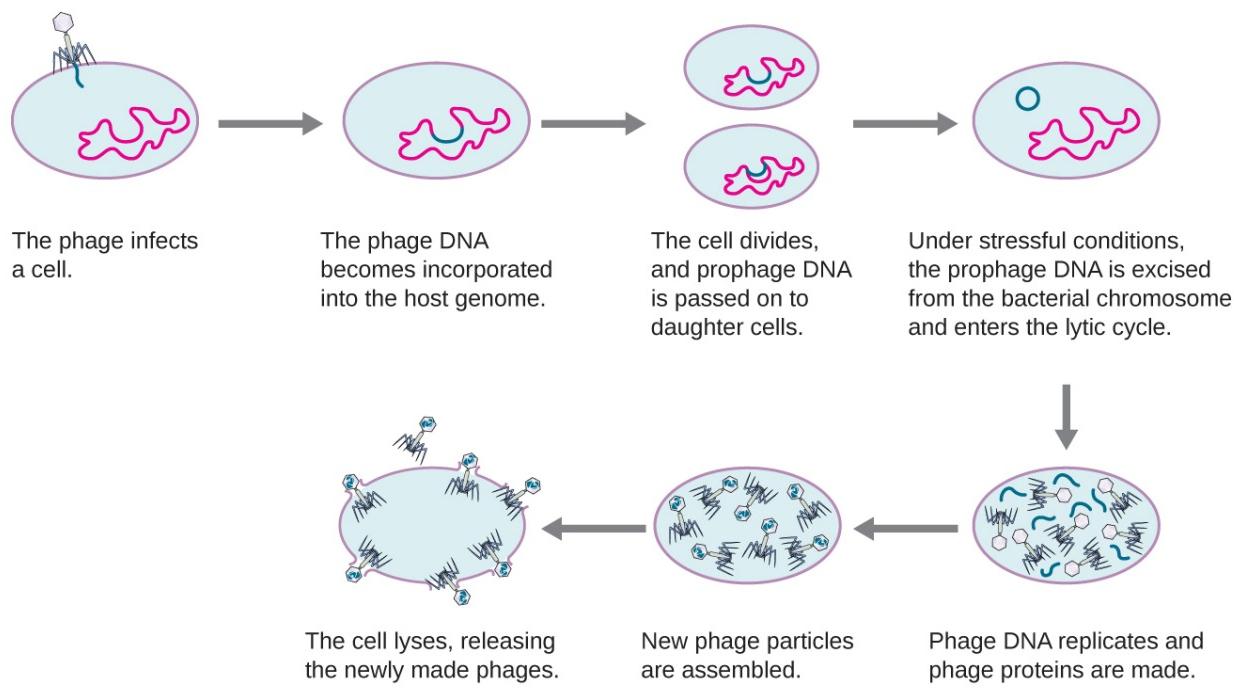
cycle, the phage replicates and lyses the host cell.

The third stage of infection is **biosynthesis** of new viral components. After entering the host cell, the virus synthesizes virus-encoded endonucleases to degrade the bacterial chromosome. It then hijacks the host cell to replicate, transcribe, and translate the necessary viral components (capsomeres, sheath, base plates, tail fibers, and viral enzymes) for the assembly of new viruses. Polymerase genes are usually expressed early in the cycle, while capsid and tail proteins are expressed later. During the **maturation** phase, new virions are created. To liberate free phages, the bacterial cell wall is disrupted by phage proteins such as holin or lysozyme. The final stage is **release**. Mature viruses can either burst out of the host cell in a process called **lysis** when progeny viruses are liberated it into the environment to infect new cells.

The Lysogenic Cycle

In a **lysogenic cycle**, the phage genome also enters the cell through attachment and penetration. A prime example of a phage with this type of life cycle is the lambda phage. During the lysogenic cycle, instead of killing the host, the phage genome integrates into the bacterial chromosome and becomes part of the host. The integrated phage genome is called a **prophage**. A bacterial host with a prophage is called a **lysogen**. The process in which a bacterium is infected by a temperate phage is called **lysogeny**. It is typical of temperate phages to be latent or inactive within the cell. As the bacterium replicates its chromosome, it also replicates the phage's DNA and passes it on to new daughter cells during reproduction. The presence of the phage may alter the phenotype of the bacterium, since it can bring in extra genes (e.g., toxin genes that can increase bacterial virulence). This change in the host phenotype is called **lysogenic conversion or phage conversion**. Some bacteria, such as *Vibrio cholerae* and *Clostridium botulinum*, are less virulent in the absence of the prophage. The phages infecting these bacteria carry the toxin genes in their genome and enhance the virulence of the host when the toxin genes are expressed. In the case of

V. cholera, phage encoded toxin can cause severe diarrhea; in *C. botulinum*, the toxin can cause paralysis. During lysogeny, the prophage will persist in the host chromosome until **induction**, which results in the excision of the viral genome from the host chromosome. After induction has occurred the temperate phage can proceed through a lytic cycle and then undergo lysogeny in a newly infected cell (see [[link](#)]).



A temperate bacteriophage has both lytic and lysogenic cycles. In the lysogenic cycle, phage DNA is incorporated into the host genome, forming a prophage, which is passed on to subsequent generations of cells. Environmental stressors such as starvation or exposure to toxic chemicals may cause the prophage to be excised and enter the lytic cycle.

Note:



This [video](#) illustrates the stages of the lysogenic life cycle of a bacteriophage and the transition to a lytic phase.

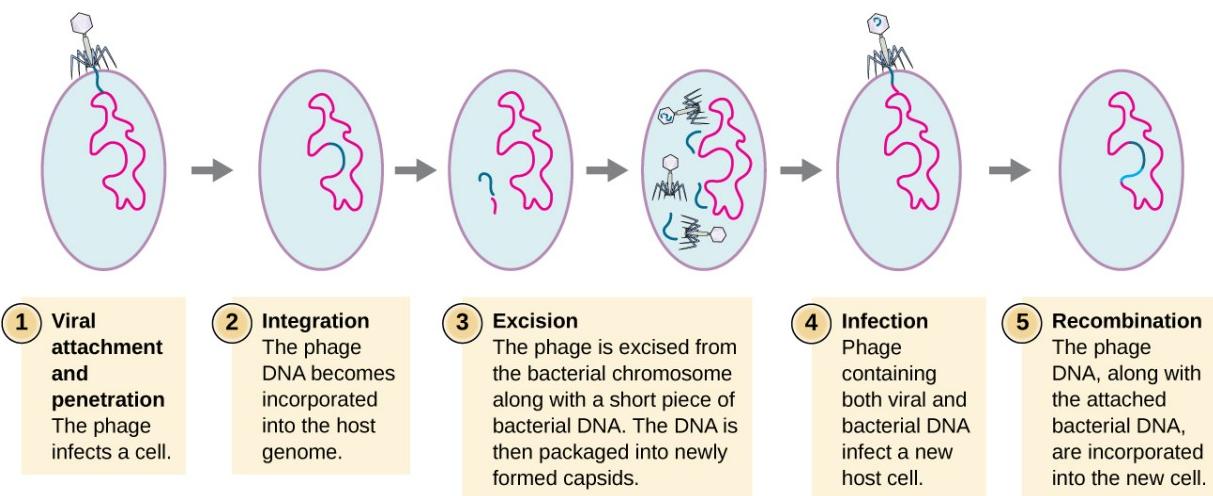
Note:

- Is a latent phage undetectable in a bacterium?

Transduction

Transduction occurs when a bacteriophage transfers bacterial DNA from one bacterium to another during sequential infections. There are two types of transduction: generalized and specialized transduction. During the lytic cycle of viral replication, the virus hijacks the host cell, degrades the host chromosome, and makes more viral genomes. As it assembles and packages DNA into the phage head, packaging occasionally makes a mistake. Instead of packaging viral DNA, it takes a random piece of host DNA and inserts it into the capsid. Once released, this virion will then inject the former host's DNA into a newly infected host. The asexual transfer of genetic information can allow for DNA recombination to occur, thus providing the new host with new genes (e.g., an antibiotic-resistance gene, or a sugar-metabolizing gene). **Generalized transduction** occurs when a random piece of bacterial chromosomal DNA is transferred by the phage during the lytic cycle. **Specialized transduction** occurs at the end of the lysogenic cycle, when the prophage is excised and the bacteriophage enters the lytic cycle. Since the phage is integrated into the host genome, the prophage can

replicate as part of the host. However, some conditions (e.g., ultraviolet light exposure or chemical exposure) stimulate the prophage to undergo induction, causing the phage to excise from the genome, enter the lytic cycle, and produce new phages to leave host cells. During the process of excision from the host chromosome, a phage may occasionally remove some bacterial DNA near the site of viral integration. The phage and host DNA from one end or both ends of the integration site are packaged within the capsid and are transferred to the new, infected host. Since the DNA transferred by the phage is not randomly packaged but is instead a specific piece of DNA near the site of integration, this mechanism of gene transfer is referred to as specialized transduction (see [\[link\]](#)). The DNA can then recombine with host chromosome, giving the latter new characteristics. Transduction seems to play an important role in the evolutionary process of bacteria, giving them a mechanism for asexual exchange of genetic information.



This flowchart illustrates the mechanism of specialized transduction.

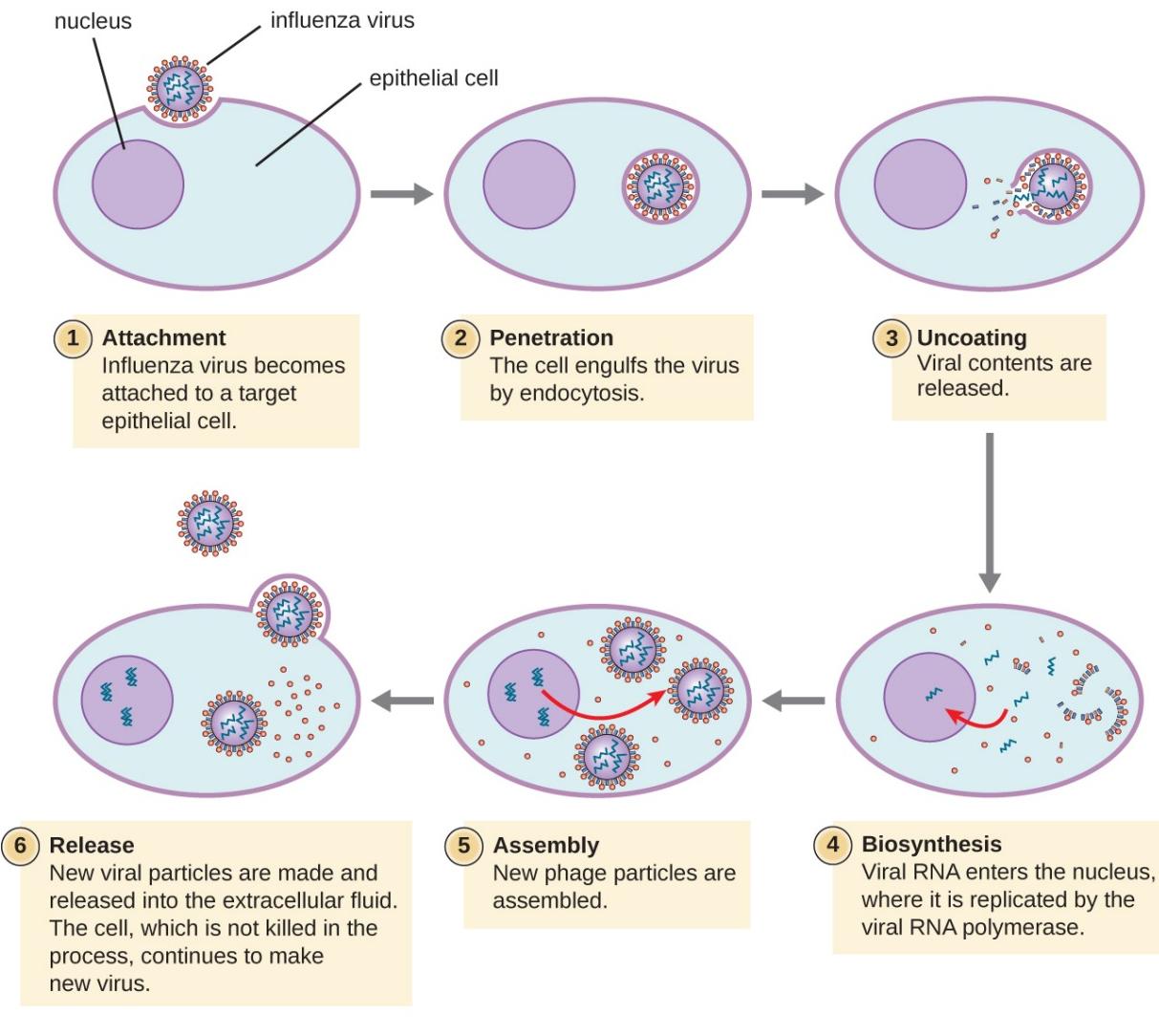
An integrated phage excises, bringing with it a piece of the DNA adjacent to its insertion point. On reinfection of a new bacterium, the phage DNA integrates along with the genetic material acquired from the previous host.

Note:

- Which phage life cycle is associated with which forms of transduction?

Life Cycle of Viruses with Animal Hosts

Lytic animal viruses follow similar infection stages to bacteriophages: attachment, penetration, biosynthesis, maturation, and release (see [[link](#)]). However, the mechanisms of penetration, nucleic-acid biosynthesis, and release differ between bacterial and animal viruses. After binding to host receptors, animal viruses enter through endocytosis (engulfment by the host cell) or through membrane fusion (viral envelope with the host cell membrane). Many viruses are host specific, meaning they only infect a certain type of host; and most viruses only infect certain types of cells within tissues. This specificity is called a **tissue tropism**. Examples of this are demonstrated by the poliovirus, which exhibits tropism for the tissues of the brain and spinal cord, or the influenza virus, which has a primary tropism for the respiratory tract.

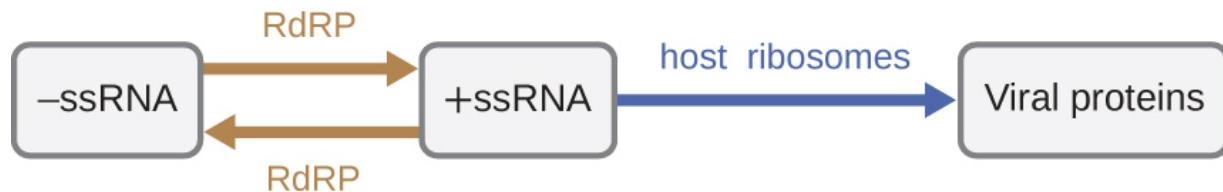


In influenza virus infection, viral glycoproteins attach the virus to a host epithelial cell. As a result, the virus is engulfed. Viral RNA and viral proteins are made and assembled into new virions that are released by budding.

Animal viruses do not always express their genes using the normal flow of genetic information—from DNA to RNA to protein. Some viruses have a dsDNA genome like cellular organisms and can follow the normal flow. However, others may have ssDNA, dsRNA, or ssRNA genomes. The nature of the genome determines how the genome is replicated and expressed as viral proteins. If a genome is ssDNA, host enzymes will be used to

synthesize a second strand that is complementary to the genome strand, thus producing dsDNA. The dsDNA can now be replicated, transcribed, and translated similar to host DNA.

If the viral genome is RNA, a different mechanism must be used. There are three types of RNA genome: dsRNA, **positive (+) single-strand (+ssRNA)** or **negative (-) single-strand RNA (-ssRNA)**. If a virus has a +ssRNA genome, it can be translated directly to make viral proteins. Viral genomic +ssRNA acts like cellular mRNA. However, if a virus contains a -ssRNA genome, the host ribosomes cannot translate it until the -ssRNA is replicated into +ssRNA by viral RNA-dependent RNA polymerase (RdRP) (see [\[link\]](#)). The RdRP is brought in by the virus and can be used to make +ssRNA from the original -ssRNA genome. The RdRP is also an important enzyme for the replication of dsRNA viruses, because it uses the negative strand of the double-stranded genome as a template to create +ssRNA. The newly synthesized +ssRNA copies can then be translated by cellular ribosomes.



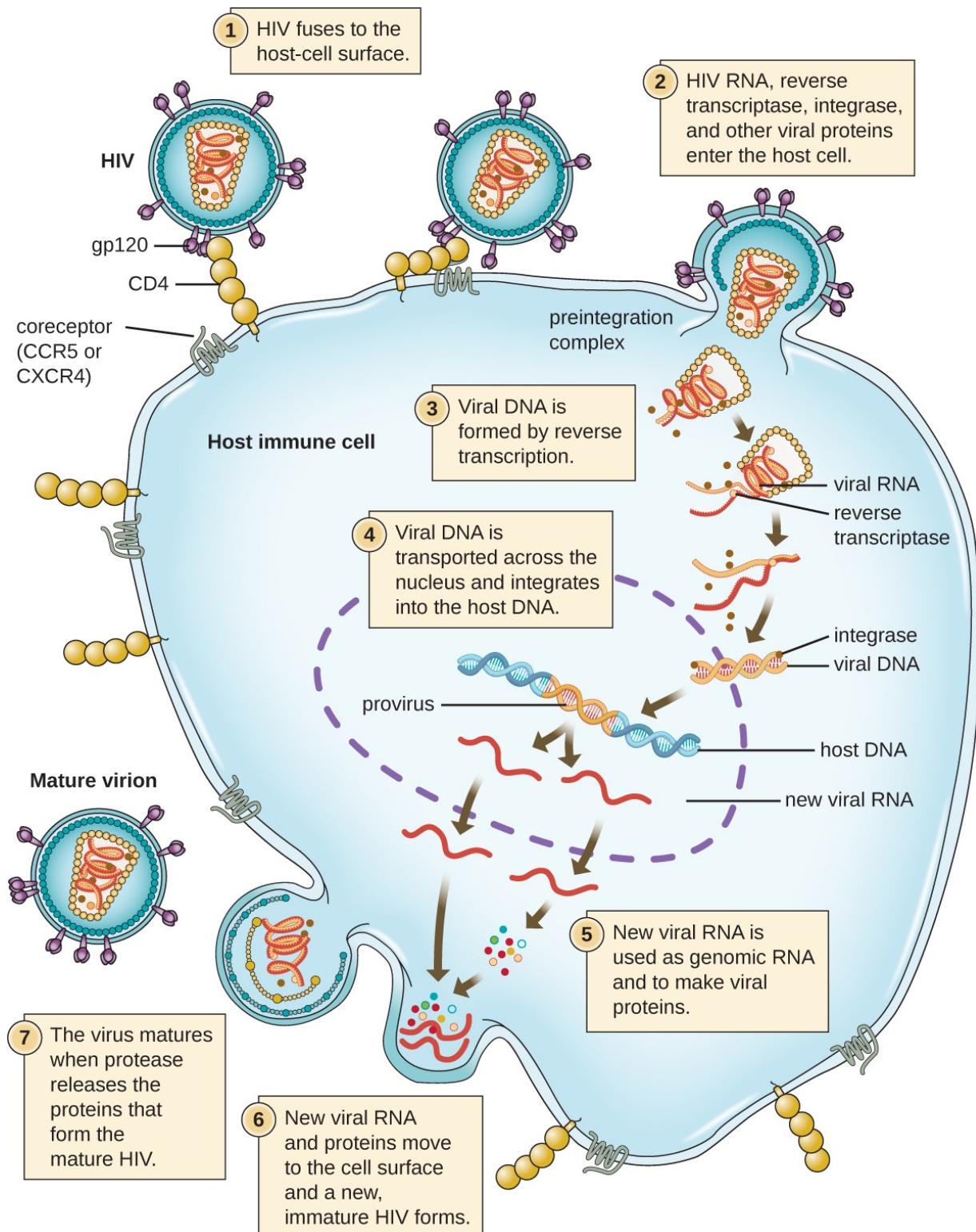
RdRP = viral RNA-dependent RNA polymerase

+ssRNA = positive (+) single strand

-ssRNA = negative (-) single-strand RNA

RNA viruses can contain +ssRNA that can be directly read by the ribosomes to synthesize viral proteins. Viruses containing -ssRNA must first use the -ssRNA as a template for the synthesis of +ssRNA before viral proteins can be synthesized.

An alternative mechanism for viral nucleic acid synthesis is observed in the **retroviruses**, which are +ssRNA viruses (see [[link](#)]). Single-stranded RNA viruses such as HIV carry a special enzyme called **reverse transcriptase** within the capsid that synthesizes a complementary ssDNA (cDNA) copy using the +ssRNA genome as a template. The ssDNA is then made into dsDNA, which can integrate into the host chromosome and become a permanent part of the host. The integrated viral genome is called a **provirus**. The virus now can remain in the host for a long time to establish a chronic infection. The provirus stage is similar to the prophage stage in a bacterial infection during the lysogenic cycle. However, unlike prophage, the provirus does not undergo excision after splicing into the genome.



HIV, an enveloped, icosahedral retrovirus, attaches to a cell surface receptor of an immune cell and fuses with the cell membrane. Viral contents are released into the cell, where viral enzymes convert the

single-stranded RNA genome into DNA and incorporate it into the host genome. (credit: modification of work by NIAID, NIH)

Note:

- Is RNA-dependent RNA polymerase made from a viral gene or a host gene?

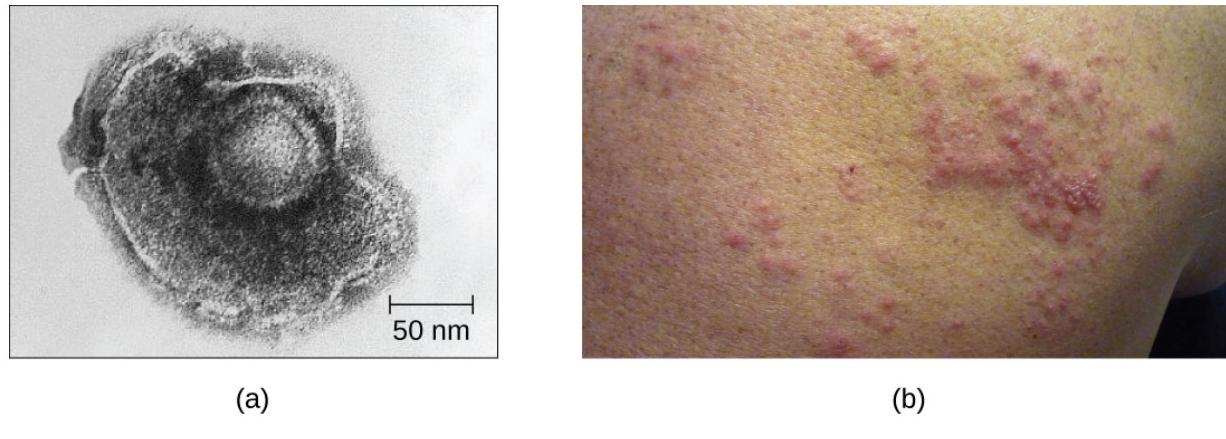
Persistent Infections

Persistent infection occurs when a virus is not completely cleared from the system of the host but stays in certain tissues or organs of the infected person. The virus may remain silent or undergo productive infection without seriously harming or killing the host. Mechanisms of persistent infection may involve the regulation of the viral or host gene expressions or the alteration of the host immune response. The two primary categories of persistent infections are latent infection and chronic infection. Examples of viruses that cause latent infections include herpes simplex virus (oral and genital herpes), varicella-zoster virus (chickenpox and shingles), and Epstein-Barr virus (mononucleosis). Hepatitis C virus and HIV are two examples of viruses that cause long-term chronic infections.

Latent Infection

Not all animal viruses undergo replication by the lytic cycle. There are viruses that are capable of remaining hidden or dormant inside the cell in a process called latency. These types of viruses are known as **latent viruses** and may cause latent infections. Viruses capable of latency may initially cause an acute infection before becoming dormant.

For example, the varicella-zoster virus infects many cells throughout the body and causes chickenpox, characterized by a rash of blisters covering the skin. About 10 to 12 days postinfection, the disease resolves and the virus goes dormant, living within nerve-cell ganglia for years. During this time, the virus does not kill the nerve cells or continue replicating. It is not clear why the virus stops replicating within the nerve cells and expresses few viral proteins but, in some cases, typically after many years of dormancy, the virus is reactivated and causes a new disease called shingles ([\[link\]](#)). Whereas chickenpox affects many areas throughout the body, shingles is a nerve cell-specific disease emerging from the ganglia in which the virus was dormant.



(a) Varicella-zoster, the virus that causes chickenpox, has an enveloped icosahedral capsid visible in this transmission electron micrograph. Its double-stranded DNA genome becomes incorporated in the host DNA.

(b) After a period of latency, the virus can reactivate in the form of shingles, usually manifesting as a painful, localized rash on one side of the body. (credit a: modification of work by Erskine Palmer and B.G. Partin—scale-bar data from Matt Russell; credit b: modification of work by Rosmarie Voegtli)

Latent viruses may remain dormant by existing as circular viral genome molecules outside of the host chromosome. Others become proviruses by

integrating into the host genome. During dormancy, viruses do not cause any symptoms of disease and may be difficult to detect. A patient may be unaware that he or she is carrying the virus unless a viral diagnostic test has been performed.

Chronic Infection

A chronic infection is a disease with symptoms that are recurrent or persistent over a long time. Some viral infections can be chronic if the body is unable to eliminate the virus. HIV is an example of a virus that produces a chronic infection, often after a long period of latency. Once a person becomes infected with HIV, the virus can be detected in tissues continuously thereafter, but untreated patients often experience no symptoms for years. However, the virus maintains chronic persistence through several mechanisms that interfere with immune function, including preventing expression of viral antigens on the surface of infected cells, altering immune cells themselves, restricting expression of viral genes, and rapidly changing viral antigens through mutation. Eventually, the damage to the immune system results in progression of the disease leading to acquired immunodeficiency syndrome (AIDS). The various mechanisms that HIV uses to avoid being cleared by the immune system are also used by other chronically infecting viruses, including the hepatitis C virus.

Note:

- In what two ways can a virus manage to maintain a persistent infection?

Life Cycle of Viruses with Plant Hosts

Plant viruses are more similar to animal viruses than they are to bacteriophages. Plant viruses may be enveloped or non-enveloped. Like many animal viruses, plant viruses can have either a DNA or RNA genome and be single stranded or double stranded. However, most plant viruses do not have a DNA genome; the majority have a +ssRNA genome, which acts like messenger RNA (mRNA). Only a minority of plant viruses have other types of genomes.

Plant viruses may have a narrow or broad host range. For example, the citrus tristeza virus infects only a few plants of the *Citrus* genus, whereas the cucumber mosaic virus infects thousands of plants of various plant families. Most plant viruses are transmitted by contact between plants, or by fungi, nematodes, insects, or other arthropods that act as mechanical vectors. However, some viruses can only be transferred by a specific type of insect vector; for example, a particular virus might be transmitted by aphids but not whiteflies. In some cases, viruses may also enter healthy plants through wounds, as might occur due to pruning or weather damage.

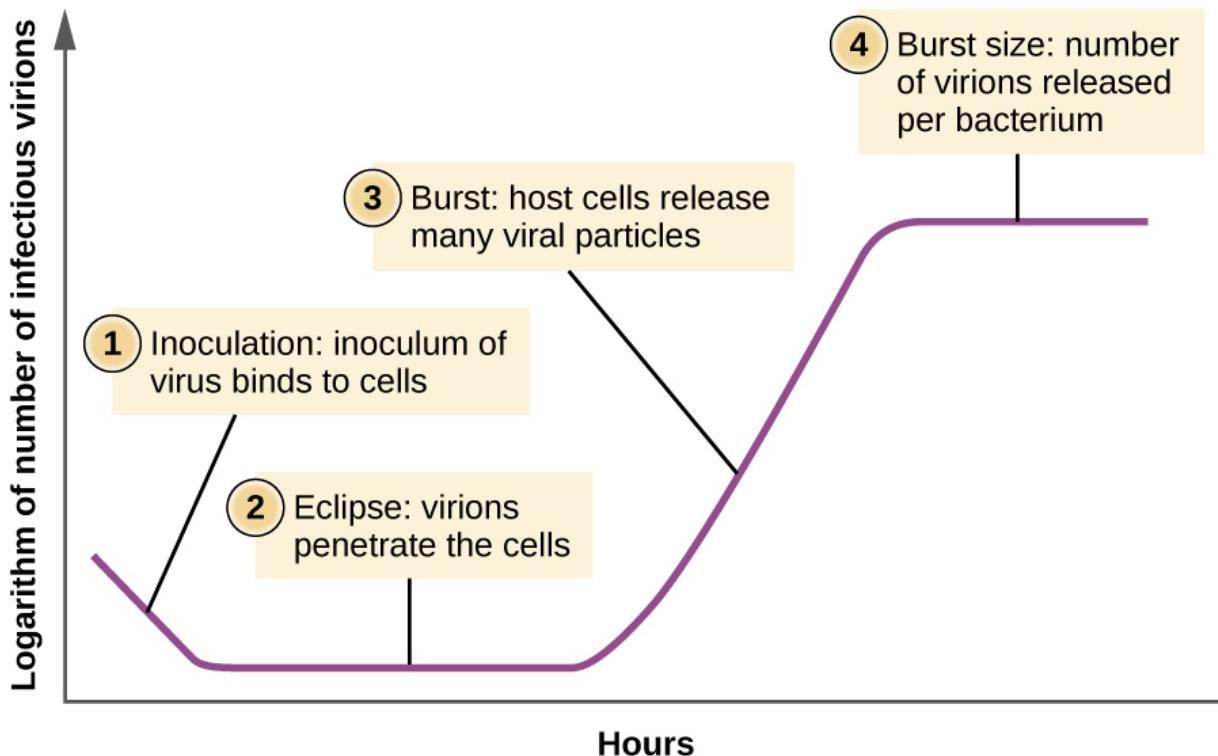
Viruses that infect plants are considered biotrophic parasites, which means that they can establish an infection without killing the host, similar to what is observed in the lysogenic life cycles of bacteriophages. Viral infection can be asymptomatic (latent) or can lead to cell death (lytic infection). The life cycle begins with the penetration of the virus into the host cell. Next, the virus is uncoated within the cytoplasm of the cell when the capsid is removed. Depending on the type of nucleic acid, cellular components are used to replicate the viral genome and synthesize viral proteins for assembly of new virions. To establish a systemic infection, the virus must enter a part of the vascular system of the plant, such as the phloem. The time required for systemic infection may vary from a few days to a few weeks depending on the virus, the plant species, and the environmental conditions. The virus life cycle is complete when it is transmitted from an infected plant to a healthy plant.

Note:

- What is the structure and genome of a typical plant virus?

Viral Growth Curve

Unlike the growth curve for a bacterial population, the growth curve for a virus population over its life cycle does not follow a sigmoidal curve. During the initial stage, an inoculum of virus causes infection. In the **eclipse phase**, viruses bind and penetrate the cells with no virions detected in the medium. The chief difference that next appears in the viral growth curve compared to a bacterial growth curve occurs when virions are released from the lysed host cell at the same time. Such an occurrence is called a **burst**, and the number of virions per bacterium released is described as the **burst size**. In a one-step multiplication curve for bacteriophage, the host cells lyse, releasing many viral particles to the medium, which leads to a very steep rise in **viral titer** (the number of virions per unit volume). If no viable host cells remain, the viral particles begin to degrade during the decline of the culture (see [[link](#)]).



The one-step multiplication curve for a bacteriophage population follows three steps: 1) inoculation, during which the virions attach to host cells; 2) eclipse, during which entry of the viral genome occurs; and 3) burst, when sufficient numbers of new virions are produced and emerge from the host cell. The burst size is the maximum number of virions produced per bacterium.

Note:

- What aspect of the life cycle of a virus leads to the sudden increase in the growth curve?

Note:

Unregistered Treatments

Ebola is incurable and deadly. The outbreak in West Africa in 2014 was unprecedented, dwarfing other human Ebola epidemics in the level of mortality. Of 24,666 suspected or confirmed cases reported, 10,179 people died.[\[footnote\]](#)

World Health Organization. “WHO Ebola Data and Statistics.” March 18, 2005. <http://apps.who.int/gho/data/view.ebola-sitrep.ebola-summary-20150318?lang=en>

No approved treatments or vaccines for Ebola are available. While some drugs have shown potential in laboratory studies and animal models, they have not been tested in humans for safety and effectiveness. Not only are these drugs untested or unregistered but they are also in short supply.

Given the great suffering and high mortality rates, it is fair to ask whether unregistered and untested medications are better than none at all. Should such drugs be dispensed and, if so, who should receive them, in light of their extremely limited supplies? Is it ethical to treat untested drugs on patients with Ebola? On the other hand, is it ethical to withhold potentially life-saving drugs from dying patients? Or should the drugs perhaps be reserved for health-care providers working to contain the disease?

In August 2014, two infected US aid workers and a Spanish priest were treated with ZMapp, an unregistered drug that had been tested in monkeys but not in humans. The two American aid workers recovered, but the priest died. Later that month, the WHO released a report on the ethics of treating patients with the drug. Since Ebola is often fatal, the panel reasoned that it is ethical to give the unregistered drugs and unethical to withhold them for safety concerns. This situation is an example of “compassionate use” outside the well-established system of regulation and governance of therapies.

Note:

Ebola in the US

On September 24, 2014, Thomas Eric Duncan arrived at the Texas Health Presbyterian Hospital in Dallas complaining of a fever, headache, vomiting, and diarrhea—symptoms commonly observed in patients with the cold or the flu. After examination, an emergency department doctor

diagnosed him with sinusitis, prescribed some antibiotics, and sent him home. Two days later, Duncan returned to the hospital by ambulance. His condition had deteriorated and additional blood tests confirmed that he has been infected with the Ebola virus.

Further investigations revealed that Duncan had just returned from Liberia, one of the countries in the midst of a severe Ebola epidemic. On September 15, nine days before he showed up at the hospital in Dallas, Duncan had helped transport an Ebola-stricken neighbor to a hospital in Liberia. The hospital continued to treat Duncan, but he died several days after being admitted.

The timeline of the Duncan case is indicative of the life cycle of the Ebola virus. The incubation time for Ebola ranges from 2 days to 21 days. Nine days passed between Duncan's exposure to the virus infection and the appearance of his symptoms. This corresponds, in part, to the eclipse period in the growth of the virus population. During the eclipse phase, Duncan would have been unable to transmit the disease to others.

However, once an infected individual begins exhibiting symptoms, the disease becomes very contagious. Ebola virus is transmitted through direct contact with droplets of bodily fluids such as saliva, blood, and vomit. Duncan could conceivably have transmitted the disease to others at any time after he began having symptoms, presumably some time before his arrival at the hospital in Dallas. Once a hospital realizes a patient like Duncan is infected with Ebola virus, the patient is immediately quarantined, and public health officials initiate a back trace to identify everyone with whom a patient like Duncan might have interacted during the period in which he was showing symptoms.

Public health officials were able to track down 10 high-risk individuals (family members of Duncan) and 50 low-risk individuals to monitor them for signs of infection. None contracted the disease. However, one of the nurses charged with Duncan's care did become infected. This, along with Duncan's initial misdiagnosis, made it clear that US hospitals needed to provide additional training to medical personnel to prevent a possible Ebola outbreak in the US.



Researchers working with Ebola virus use layers of defenses against accidental infection, including protective clothing, breathing systems, and negative air-pressure cabinets for bench work. (credit: modification of work by Randal J. Schoepp)

Note:



For additional information about Ebola, please visit the [CDC](#) website.

Key Concepts and Summary

- Many viruses target specific hosts or tissues. Some may have more than one host.
- Many viruses follow several stages to infect host cells. These stages include **attachment, penetration, uncoating, biosynthesis, maturation, and release**.
- Bacteriophages have a **lytic or lysogenic cycle**. The lytic cycle leads to the death of the host, whereas the lysogenic cycle leads to integration of phage into the host genome.
- Bacteriophages inject DNA into the host cell, whereas animal viruses enter by endocytosis or membrane fusion.
- Animal viruses can undergo **latency**, similar to lysogeny for a bacteriophage.
- The majority of plant viruses are positive-strand ssRNA and can undergo latency, chronic, or lytic infection, as observed for animal viruses.
- The growth curve of bacteriophage populations is a **one-step multiplication curve** and not a sigmoidal curve, as compared to the bacterial growth curve.
- Bacteriophages transfer genetic information between hosts using either **generalized or specialized transduction**.

Short Answer

Exercise:

Problem:

Briefly explain the difference between the mechanism of entry of a T-even bacteriophage and an animal virus.

Exercise:

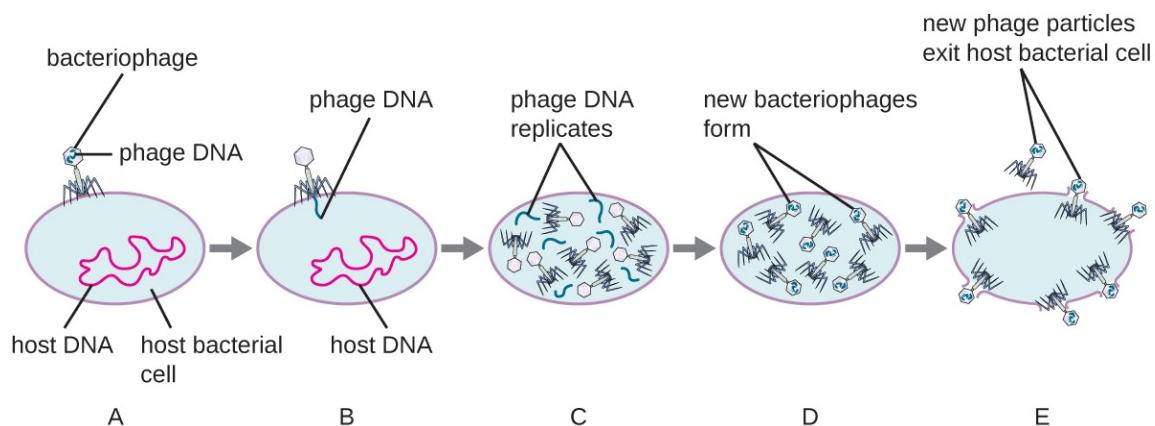
Problem: Differentiate between lytic and lysogenic cycles.

Critical Thinking

Exercise:

Problem:

Label the five stages of a bacteriophage infection in the figure:



Exercise:

Problem:

Bacteriophages have lytic and lysogenic cycles. Discuss the advantages and disadvantages for the phage.

Exercise:

Problem:

How does reverse transcriptase aid a retrovirus in establishing a chronic infection?

Exercise:

Problem:

Discuss some methods by which plant viruses are transmitted from a diseased plant to a healthy one.

Isolation, Culture, and Identification of Viruses

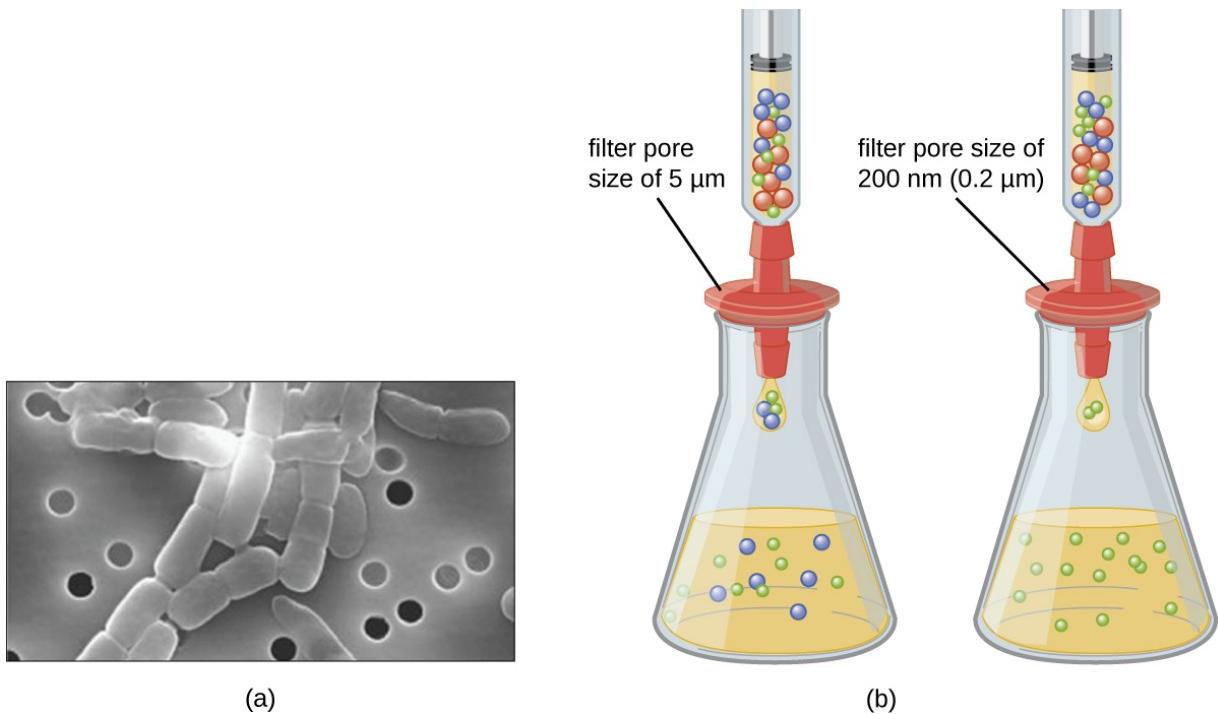
LEARNING OBJECTIVES

- Discuss why viruses were originally described as filterable agents
- Describe the cultivation of viruses and specimen collection and handling
- Compare *in vivo* and *in vitro* techniques used to cultivate viruses

At the beginning of this chapter, we described how porcelain Chamberland filters with pores small enough to allow viruses to pass through were used to discover TMV. Today, porcelain filters have been replaced with membrane filters and other devices used to isolate and identify viruses.

Isolation of Viruses

Unlike bacteria, many of which can be grown on an artificial nutrient medium, viruses require a living host cell for replication. Infected host cells (eukaryotic or prokaryotic) can be cultured and grown, and then the growth medium can be harvested as a source of virus. Virions in the liquid medium can be separated from the host cells by either centrifugation or filtration. Filters can physically remove anything present in the solution that is larger than the virions; the viruses can then be collected in the filtrate (see [\[link\]](#)).



Membrane filters can be used to remove cells or viruses from a solution. (a) This scanning electron micrograph shows rod-shaped bacterial cells captured on the surface of a membrane filter. Note differences in the comparative size of the membrane pores and bacteria. Viruses will pass through this filter. (b) The size of the pores in the filter determines what is captured on the surface of the filter (animal [red] and bacteria [blue]) and removed from liquid passing through. Note the viruses (green) pass through the finer filter. (credit a: modification of work by U.S. Department of Energy)

Note:

- What size filter pore is needed to collect a virus?

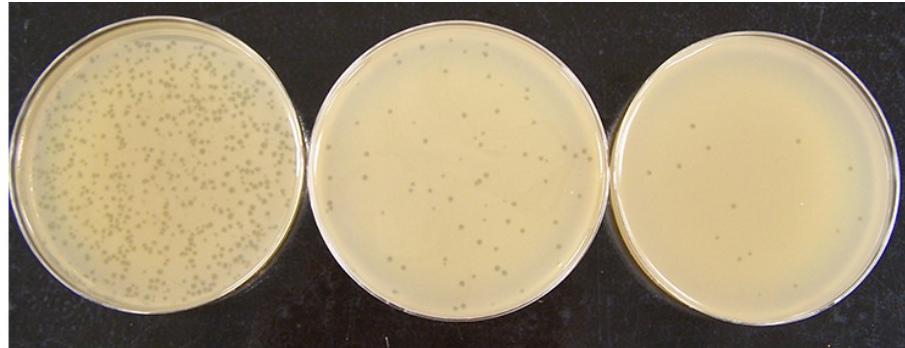
Cultivation of Viruses

Viruses can be grown **in vivo** (within a whole living organism, plant, or animal) or **in vitro** (outside a living organism in cells in an artificial environment, such as a test tube, cell culture flask, or agar plate).

Bacteriophages can be grown in the presence of a dense layer of bacteria (also called a **bacterial lawn**) grown in a 0.7 % soft agar in a Petri dish or flat (horizontal) flask (see [[link](#)]). The agar concentration is decreased from the 1.5% usually used in culturing bacteria. The soft 0.7% agar allows the bacteriophages to easily diffuse through the medium. For lytic bacteriophages, lysing of the bacterial hosts can then be readily observed when a clear zone called a **plaque** is detected (see [[link](#)]). As the phage kills the bacteria, many plaques are observed among the cloudy bacterial lawn.



(a)



(b)

(a) Flasks like this may be used to culture human or animal cells for viral culturing. (b) These plates contain bacteriophage T4 grown on an *Escherichia coli* lawn. Clear plaques are visible where host bacterial cells have been lysed. Viral titers increase on the plates to the left.
(credit a: modification of work by National Institutes of Health; credit b: modification of work by American Society for Microbiology)

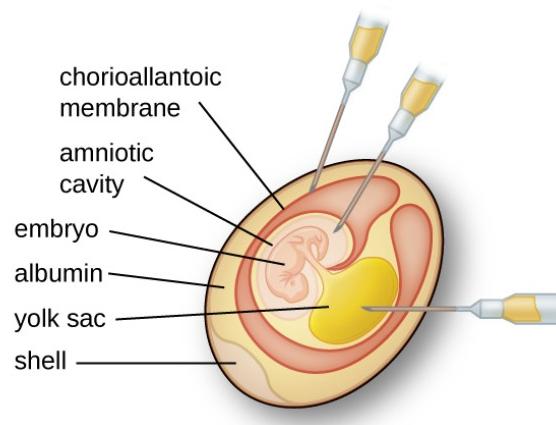
Animal viruses require cells within a host animal or tissue-culture cells derived from an animal. Animal virus cultivation is important for 1)

identification and diagnosis of pathogenic viruses in clinical specimens, 2) production of vaccines, and 3) basic research studies. In vivo host sources can be a developing embryo in an embryonated bird's egg (e.g., chicken, turkey) or a whole animal. For example, most of the influenza vaccine manufactured for annual flu vaccination programs is cultured in hens' eggs.

The embryo or host animal serves as an incubator for viral replication (see [\[link\]](#)). Location within the embryo or host animal is important. Many viruses have a tissue tropism, and must therefore be introduced into a specific site for growth. Within an embryo, target sites include the amniotic cavity, the chorioallantoic membrane, or the yolk sac. Viral infection may damage tissue membranes, producing lesions called pox; disrupt embryonic development; or cause the death of the embryo.



(a)

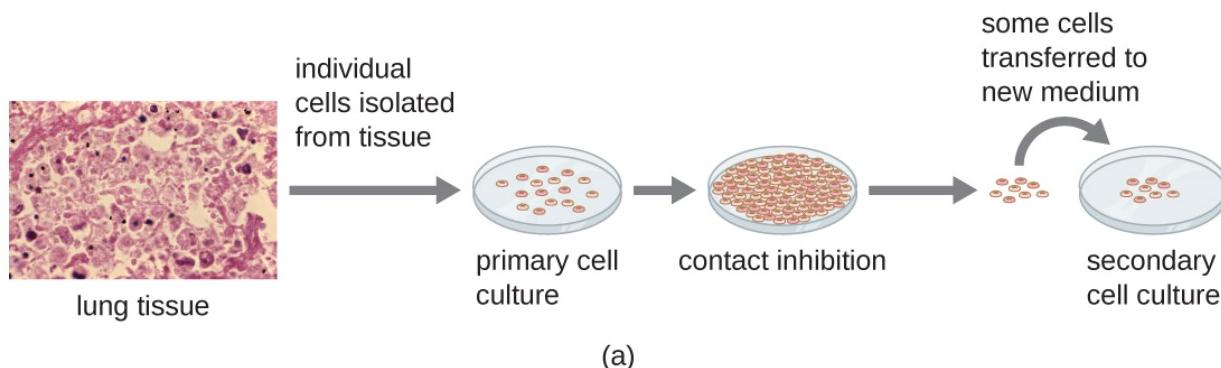


(b)

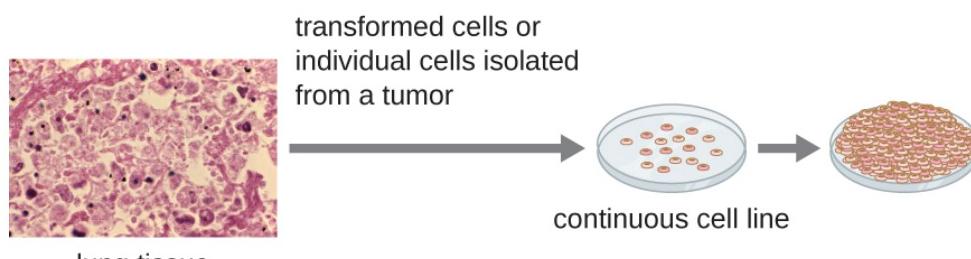
- (a) The cells within chicken eggs are used to culture different types of viruses. (b) Viruses can be replicated in various locations within the egg, including the chorioallantoic membrane, the amniotic cavity, and the yolk sac. (credit a: modification of work by “Chung Hoang”/YouTube)

For in vitro studies, various types of cells can be used to support the growth of viruses. A primary cell culture is freshly prepared from animal organs or

tissues. Cells are extracted from tissues by mechanical scraping or mincing to release cells or by an enzymatic method using trypsin or collagenase to break up tissue and release single cells into suspension. Because of anchorage-dependence requirements, primary cell cultures require a liquid culture medium in a Petri dish or tissue-culture flask so cells have a solid surface such as glass or plastic for attachment and growth. Primary cultures usually have a limited life span. When cells in a primary culture undergo mitosis and a sufficient density of cells is produced, cells come in contact with other cells. When this cell-to-cell-contact occurs, mitosis is triggered to stop. This is called contact inhibition and it prevents the density of the cells from becoming too high. To prevent contact inhibition, cells from the primary cell culture must be transferred to another vessel with fresh growth medium. This is called a secondary cell culture. Periodically, cell density must be reduced by pouring off some cells and adding fresh medium to provide space and nutrients to maintain cell growth. In contrast to primary cell cultures, continuous cell lines, usually derived from transformed cells or tumors, are often able to be subcultured many times or even grown indefinitely (in which case they are called immortal). Continuous cell lines may not exhibit anchorage dependency (they will grow in suspension) and may have lost their contact inhibition. As a result, continuous cell lines can grow in piles or lumps resembling small tumor growths (see [\[link\]](#)).



(a)



(b)

Cells for culture are prepared by separating them from their tissue matrix. (a) Primary cell cultures grow attached to the surface of the culture container. Contact inhibition slows the growth of the cells once they become too dense and begin touching each other. At this point, growth can only be sustained by making a secondary culture. (b) Continuous cell cultures are not affected by contact inhibition. They continue to grow regardless of cell density. (credit “micrographs”: modification of work by Centers for Disease Control and Prevention)

An example of an immortal cell line is the HeLa cell line, which was originally cultivated from tumor cells obtained from Henrietta Lacks, a patient who died of cervical cancer in 1951. HeLa cells were the first continuous tissue-culture cell line and were used to establish tissue culture as an important technology for research in cell biology, virology, and medicine. Prior to the discovery of HeLa cells, scientists were not able to establish tissue cultures with any reliability or stability. More than six decades later, this cell line is still alive and being used for medical research. See [Eye on Ethics: The Immortal Cell Line of Henrietta Lacks](#) to read more

about this important cell line and the controversial means by which it was obtained.

Note:

- What property of cells makes periodic dilutions of primary cell cultures necessary?

Note:

The Immortal Cell Line of Henrietta Lacks

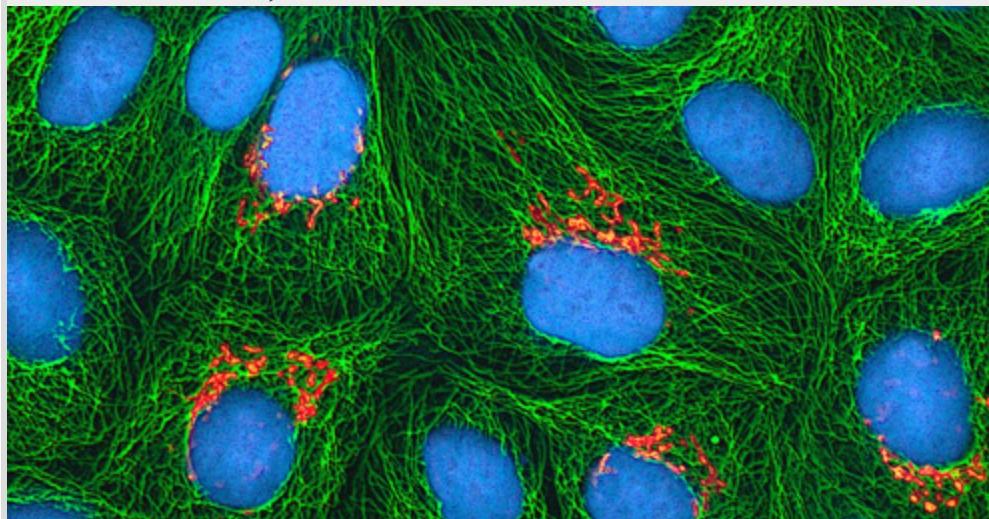
In January 1951, Henrietta Lacks, a 30-year-old African American woman from Baltimore, was diagnosed with cervical cancer at John Hopkins Hospital. We now know her cancer was caused by the human papillomavirus (HPV). Cytopathic effects of the virus altered the characteristics of her cells in a process called transformation, which gives the cells the ability to divide continuously. This ability, of course, resulted in a cancerous tumor that eventually killed Mrs. Lacks in October at age 31. Before her death, samples of her cancerous cells were taken without her knowledge or permission. The samples eventually ended up in the possession of Dr. George Gey, a biomedical researcher at Johns Hopkins University. Gey was able to grow some of the cells from Lacks's sample, creating what is known today as the immortal HeLa cell line. These cells have the ability to live and grow indefinitely and, even today, are still widely used in many areas of research.

According to Lacks's husband, neither Henrietta nor the family gave the hospital permission to collect her tissue specimen. Indeed, the family was not aware until 20 years after Lacks's death that her cells were still alive and actively being used for commercial and research purposes. Yet HeLa cells have been pivotal in numerous research discoveries related to polio, cancer, and AIDS, among other diseases. The cells have also been commercialized, although they have never themselves been patented. Despite this, Henrietta Lacks's estate has never benefited from the use of

the cells, although, in 2013, the Lacks family was given control over the publication of the genetic sequence of her cells.

This case raises several bioethical issues surrounding patients' informed consent and the right to know. At the time Lacks's tissues were taken, there were no laws or guidelines about informed consent. Does that mean she was treated fairly at the time? Certainly by today's standards, the answer would be no. Harvesting tissue or organs from a dying patient without consent is not only considered unethical but illegal, regardless of whether such an act could save other patients' lives. Is it ethical, then, for scientists to continue to use Lacks's tissues for research, even though they were obtained illegally by today's standards?

Ethical or not, Lacks's cells are widely used today for so many applications that it is impossible to list them all. Is this a case in which the ends justify the means? Would Lacks be pleased to know about her contribution to science and the millions of people who have benefited? Would she want her family to be compensated for the commercial products that have been developed using her cells? Or would she feel violated and exploited by the researchers who took part of her body without her consent? Because she was never asked, we will never know.

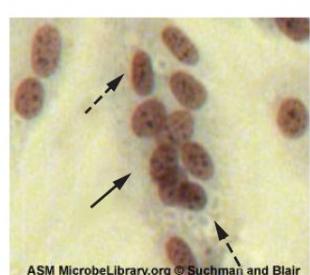
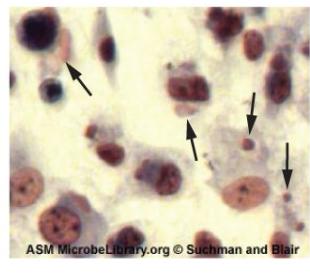
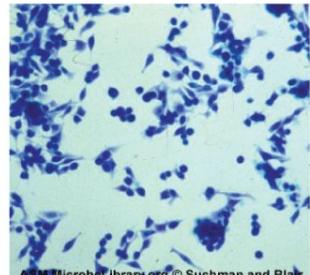


A multiphoton fluorescence image of HeLa cells in culture. Various fluorescent stains have been used to show the DNA (cyan), microtubules (green), and Golgi apparatus (orange). (credit: modification of work by National Institutes of Health)

Detection of a Virus

Regardless of the method of cultivation, once a virus has been introduced into a whole host organism, embryo, or tissue-culture cell, a sample can be prepared from the infected host, embryo, or cell line for further analysis under a brightfield, electron, or fluorescent microscope. **Cytopathic effects (CPEs)** are distinct observable cell abnormalities due to viral infection. CPEs can include loss of adherence to the surface of the container, changes in cell shape from flat to round, shrinkage of the nucleus, vacuoles in the cytoplasm, fusion of cytoplasmic membranes and the formation of multinucleated syncytia, inclusion bodies in the nucleus or cytoplasm, and complete cell lysis (see [\[link\]](#)).

Further pathological changes include viral disruption of the host genome and altering normal cells into transformed cells, which are the types of cells associated with carcinomas and sarcomas. The type or severity of the CPE depends on the type of virus involved. [\[link\]](#) lists CPEs for specific viruses.

Cytopathic Effects of Specific Viruses		
Virus	Cytopathic Effect	Example
<i>Paramyxovirus</i>	Syncytium and faint basophilic cytoplasmic inclusion bodies	 <small>ASM MicrobeLibrary.org © Suchman and Blair</small>
<i>Poxvirus</i>	Pink eosinophilic cytoplasmic inclusion bodies (arrows) and cell swelling	 <small>ASM MicrobeLibrary.org © Suchman and Blair</small>
<i>Herpesvirus</i>	Cytoplasmic stranding (arrow) and nuclear inclusion bodies (dashed arrow)	 <small>ASM MicrobeLibrary.org © Suchman and Blair</small>
<i>Adenovirus</i>	Cell enlargement, rounding, and distinctive "grape-like" clusters	 <small>ASM MicrobeLibrary.org © Suchman and Blair</small>

(credit “micrographs”: modification of work by American Society for Microbiology)

Note:

Watch this [video](#) to learn about the effects of viruses on cells.

Hemagglutination Assay

A serological assay is used to detect the presence of certain types of viruses in patient serum. Serum is the straw-colored liquid fraction of blood plasma from which clotting factors have been removed. Serum can be used in a direct assay called a hemagglutination assay to detect specific types of viruses in the patient's sample. Hemagglutination is the agglutination (clumping) together of erythrocytes (red blood cells). Many viruses produce surface proteins or spikes called hemagglutinins that can bind to receptors on the membranes of erythrocytes and cause the cells to agglutinate. Hemagglutination is observable without using the microscope, but this method does not always differentiate between infectious and noninfectious viral particles, since both can agglutinate erythrocytes.

To identify a specific pathogenic virus using hemagglutination, we must use an indirect approach. Proteins called antibodies, generated by the patient's immune system to fight a specific virus, can be used to bind to components such as hemagglutinins that are uniquely associated with specific types of viruses. The binding of the antibodies with the hemagglutinins found on the virus subsequently prevent erythrocytes from directly interacting with the virus. So when erythrocytes are added to the antibody-coated viruses, there is no appearance of agglutination; agglutination has been inhibited. We call these types of indirect assays for virus-specific antibodies hemagglutination inhibition (HAI) assays. HAI can be used to detect the presence of

antibodies specific to many types of viruses that may be causing or have caused an infection in a patient even months or years after infection (see [\[link\]](#)). This assay is described in greater detail in [Agglutination Assays](#).

	Components	Interaction	Microtiter Results
A	RBCs		No reaction
B	Virus + RBCs		Hemagglutination
C	Virus + Antibody + RBCs		Hemagglutination inhibition

This chart shows the possible outcomes of a hemagglutination test. Row A: Erythrocytes do not bind together and will sink to the bottom of the well plate; this becomes visible as a red dot in the center of the well. Row B: Many viruses have hemagglutinins that causes agglutination of erythrocytes; the resulting hemagglutination forms a lattice structure that results in red color throughout the well. Row C: Virus-specific antibody, the viruses, and the erythrocytes are added to the well plate. The virus-specific antibodies inhibit agglutination, as can be seen as a red dot in the bottom of the well. (credit: modification of work by Centers for Disease Control and Prevention)

Note:

- What is the outcome of a positive HIA test?

Nucleic Acid Amplification Test

Nucleic acid amplification tests (NAAT) are used in molecular biology to detect unique nucleic acid sequences of viruses in patient samples.

Polymerase chain reaction (PCR) is an NAAT used to detect the presence of viral DNA in a patient's tissue or body fluid sample. PCR is a technique that amplifies (i.e., synthesizes many copies) of a viral DNA segment of interest. Using PCR, short nucleotide sequences called primers bind to specific sequences of viral DNA, enabling identification of the virus.

Reverse transcriptase-PCR (RT-PCR) is an NAAT used to detect the presence of RNA viruses. RT-PCR differs from PCR in that the enzyme reverse transcriptase (RT) is used to make a cDNA from the small amount of viral RNA in the specimen. The cDNA can then be amplified by PCR. Both PCR and RT-PCR are used to detect and confirm the presence of the viral nucleic acid in patient specimens.

Note:

HPV Scare

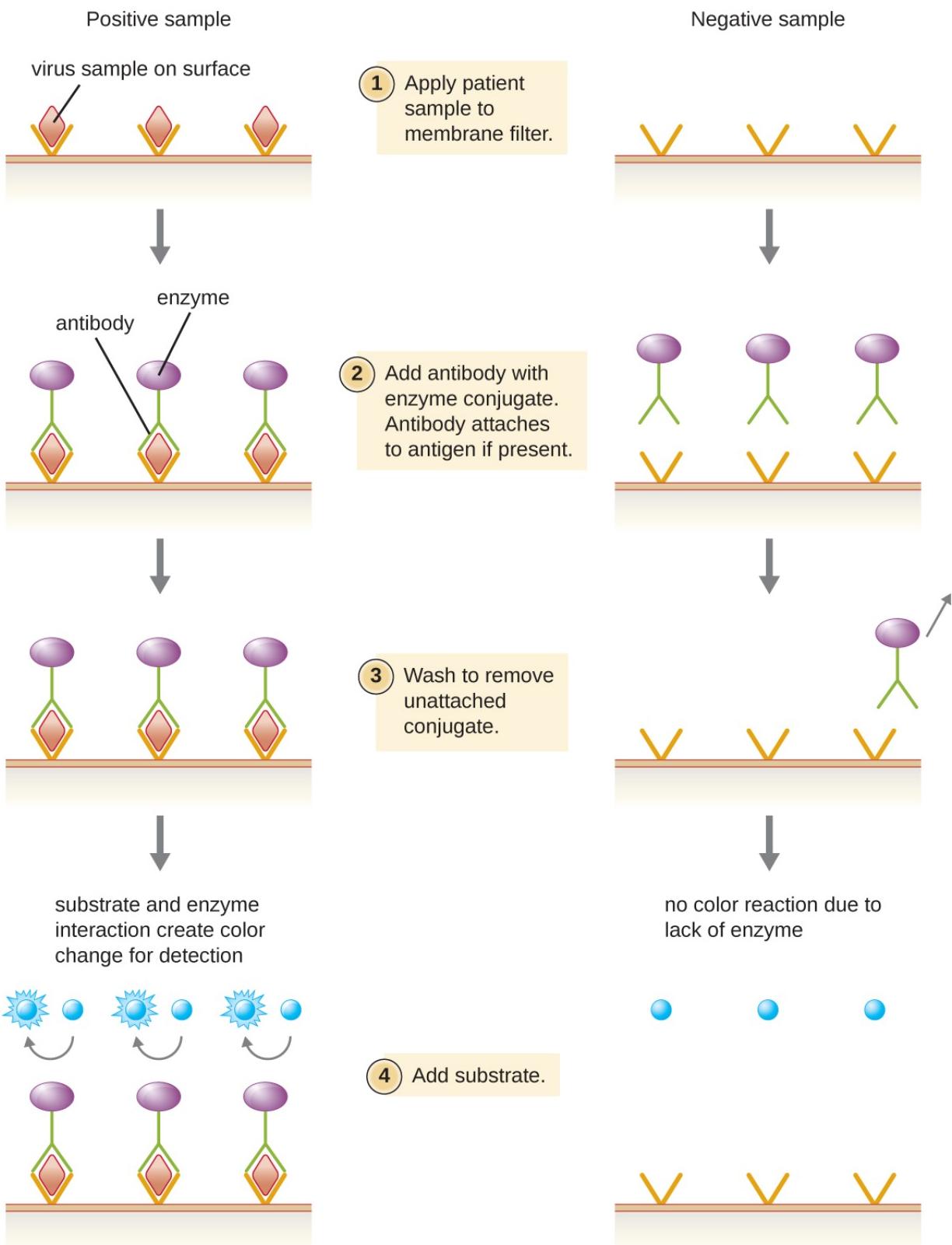
Michelle, a 21-year-old nursing student, came to the university clinic worried that she might have been exposed to a sexually transmitted disease (STD). Her sexual partner had recently developed several bumps on the base of his penis. He had put off going to the doctor, but Michelle suspects they are genital warts caused by HPV. She is especially concerned because she knows that HPV not only causes warts but is a prominent cause of cervical cancer. She and her partner always use condoms for contraception, but she is not confident that this precaution will protect her from HPV. Michelle's physician finds no physical signs of genital warts or any other STDs, but recommends that Michelle get a Pap smear along with an HPV

test. The Pap smear will screen for abnormal cervical cells and the CPEs associated with HPV; the HPV test will test for the presence of the virus. If both tests are negative, Michelle can be more assured that she most likely has not become infected with HPV. However, her doctor suggests it might be wise for Michelle to get vaccinated against HPV to protect herself from possible future exposure.

- Why does Michelle's physician order two different tests instead of relying on one or the other?

Enzyme Immunoassay

Enzyme immunoassays (EIAs) rely on the ability of antibodies to detect and attach to specific biomolecules called antigens. The detecting antibody attaches to the target antigen with a high degree of specificity in what might be a complex mixture of biomolecules. Also included in this type of assay is a colorless enzyme attached to the detecting antibody. The enzyme acts as a tag on the detecting antibody and can interact with a colorless substrate, leading to the production of a colored end product. EIAs often rely on layers of antibodies to capture and react with antigens, all of which are attached to a membrane filter (see [\[link\]](#)). EIAs for viral antigens are often used as preliminary screening tests. If the results are positive, further confirmation will require tests with even greater sensitivity, such as a western blot or an NAAT. EIAs are discussed in more detail in [EIAs and ELISAs](#).



Similar to rapid, over-the-counter pregnancy tests, EIAs for viral

antigens require a few drops of diluted patient serum or plasma applied to a membrane filter. The membrane filter has been previously modified and embedded with antibody to viral antigen and internal controls. Antibody conjugate is added to the filter, with the targeted antibody attached to the antigen (in the case of a positive test). Excess conjugate is washed off the filter. Substrate is added to activate the enzyme-mediated reaction to reveal the color change of a positive test.
(credit: modification of work by “Cavitri”/Wikimedia Commons)

Note:

- What typically indicates a positive EIA test?

Key Concepts and Summary

- Viral cultivation requires the presence of some form of host cell (whole organism, embryo, or cell culture).
- Viruses can be isolated from samples by filtration.
- Viral filtrate is a rich source of released virions.
- Bacteriophages are detected by presence of clear **plaques** on bacterial lawn.
- Animal and plant viruses are detected by **cytopathic effects**, molecular techniques (PCR, RT-PCR), enzyme immunoassays, and serological assays (hemagglutination assay, hemagglutination inhibition assay).

Short Answer

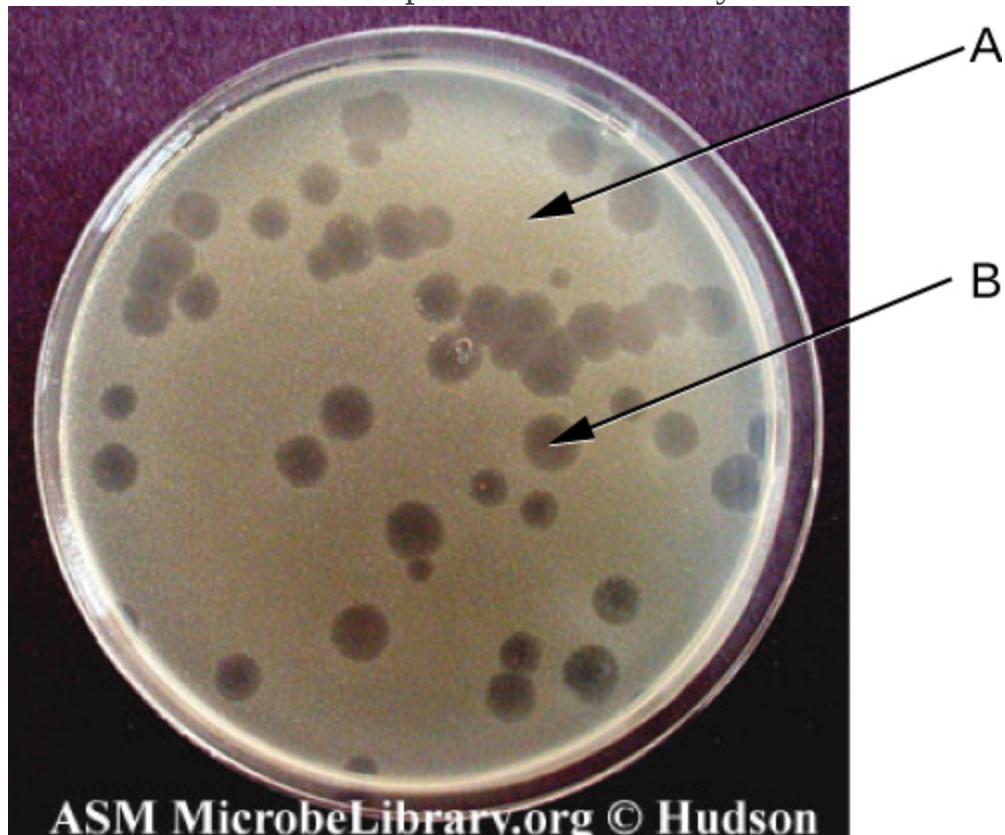
Exercise:

Problem: Briefly explain the various methods of culturing viruses.

Critical Thinking

Exercise:

Problem: Label the components indicated by arrows.



ASM MicrobeLibrary.org © Hudson

(credit: modification of work by American Society for Microbiology)

Exercise:

Problem:

What are some characteristics of the viruses that are similar to a computer virus?

Viroids, Virusoids, and Prions

LEARNING OBJECTIVES

- Describe viroids and their unique characteristics
- Describe virusoids and their unique characteristics
- Describe prions and their unique characteristics

Research attempts to discover the causative agents of previously uninvestigated diseases have led to the discovery of nonliving disease agents quite different from viruses. These include particles consisting only of RNA or only of protein that, nonetheless, are able to self-propagate at the expense of a host—a key similarity to viruses that allows them to cause disease conditions. To date, these discoveries include viroids, virusoids, and the proteinaceous prions.

Viroids

In 1971, Theodor Diener, a pathologist working at the Agriculture Research Service, discovered an acellular particle that he named a viroid, meaning “virus-like.” **Viroids** consist only of a short strand of circular RNA capable of self-replication. The first viroid discovered was found to cause potato tuber spindle disease, which causes slower sprouting and various deformities in potato plants (see [[link](#)]). Like viruses, potato spindle tuber viroids (PSTVs) take control of the host machinery to replicate their RNA genome. Unlike viruses, viroids do not have a protein coat to protect their genetic information.



These potatoes have been infected by the potato spindle tuber viroid (PSTV), which is typically spread when infected knives are used to cut healthy potatoes, which are then planted. (credit: Pamela Roberts, University of Florida Institute of Food and Agricultural Sciences, USDA ARS)

Viroids can result in devastating losses of commercially important agricultural food crops grown in fields and orchards. Since the discovery of PSTV, other viroids have been discovered that cause diseases in plants. Tomato planta macho viroid (TPMVd) infects tomato plants, which causes loss of chlorophyll, disfigured and brittle leaves, and very small tomatoes, resulting in loss of productivity in this field crop. Avocado sunblotch viroid (ASBVd) results in lower yields and poorer-quality fruit. ASBVd is the smallest viroid discovered thus far that infects plants. Peach latent mosaic viroid (PLMVD) can cause necrosis of flower buds and branches, and wounding of ripened fruit, which leads to fungal and bacterial growth in the fruit. PLMVD can also cause similar pathological changes in plums, nectarines, apricots, and cherries, resulting in decreased productivity in these orchards, as well. Viroids, in general, can be dispersed mechanically during crop maintenance or harvesting, vegetative

reproduction, and possibly via seeds and insects, resulting in a severe drop in food availability and devastating economic consequences.

Note:

- What is the genome of a viroid made of?

Virusoids

A second type of pathogenic RNA that can infect commercially important agricultural crops are the **virusoids**, which are subviral particles best described as non-self-replicating ssRNAs. RNA replication of virusoids is similar to that of viroids but, unlike viroids, virusoids require that the cell also be infected with a specific “helper” virus. There are currently only five described types of virusoids and their associated helper viruses. The helper viruses are all from the family of Sobemoviruses. An example of a helper virus is the subterranean clover mottle virus, which has an associated virusoid packaged inside the viral capsid. Once the helper virus enters the host cell, the virusoids are released and can be found free in plant cell cytoplasm, where they possess ribozyme activity. The helper virus undergoes typical viral replication independent of the activity of the virusoid. The virusoid genomes are small, only 220 to 388 nucleotides long. A virusoid genome does not code for any proteins, but instead serves only to replicate virusoid RNA.

Virusoids belong to a larger group of infectious agents called satellite RNAs, which are similar pathogenic RNAs found in animals. Unlike the plant virusoids, satellite RNAs may encode for proteins; however, like plant virusoids, satellite RNAs must coinfect with a helper virus to replicate. One satellite RNA that infects humans and that has been described by some scientists as a virusoid is the hepatitis delta virus (HDV), which, by some reports, is also called hepatitis delta virusoid. Much larger than a plant virusoid, HDV has a circular, ssRNA genome of 1,700 nucleotides and can direct the biosynthesis of HDV-associated proteins. The HDV helper virus is the hepatitis B virus (HBV). Coinfection with HBV and HDV results in more severe

pathological changes in the liver during infection, which is how HDV was first discovered.

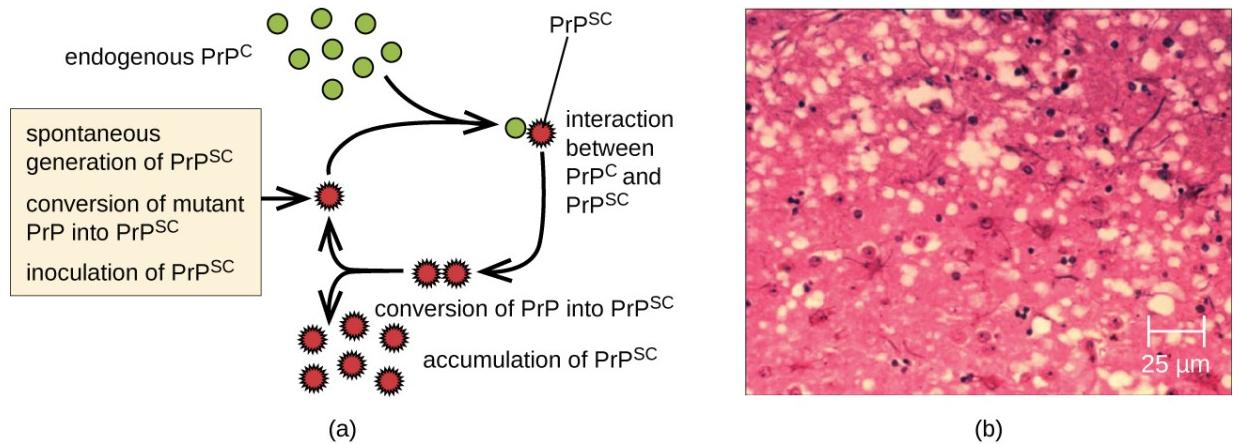
Note:

- What is the main difference between a viroid and a virusoid?

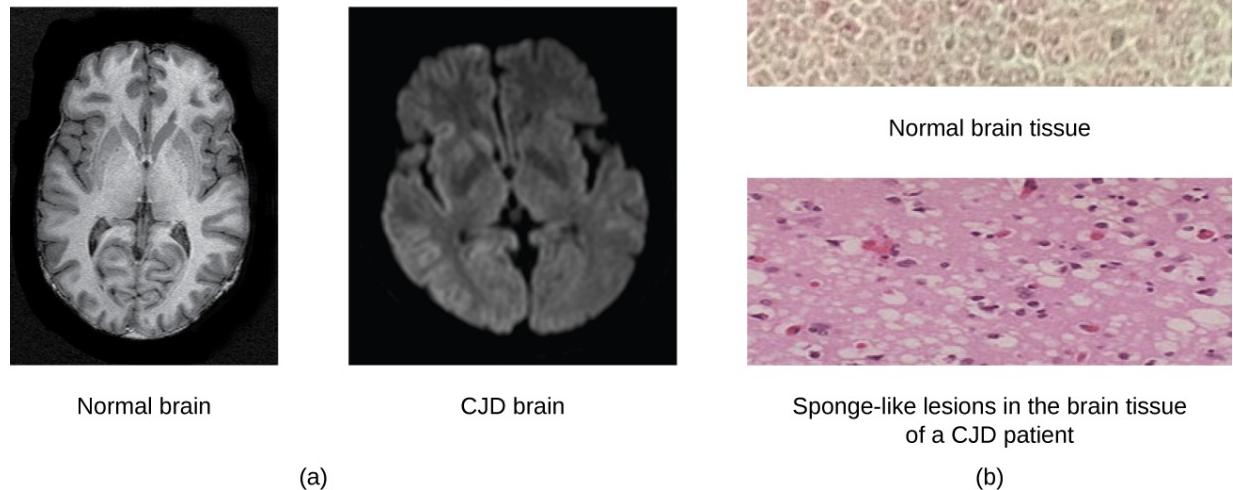
Prions

At one time, scientists believed that any infectious particle must contain DNA or RNA. Then, in 1982, Stanley Prusiner, a medical doctor studying scrapie (a fatal, degenerative disease in sheep) discovered that the disease was caused by proteinaceous infectious particles, or **prions**. Because proteins are acellular and do not contain DNA or RNA, Prusiner's findings were originally met with resistance and skepticism; however, his research was eventually validated, and he received the Nobel Prize in Physiology or Medicine in 1997.

A prion is a misfolded rogue form of a normal protein (PrP^c) found in the cell. This rogue prion protein (PrP^sc), which may be caused by a genetic mutation or occur spontaneously, can be infectious, stimulating other endogenous normal proteins to become misfolded, forming plaques (see [\[link\]](#)). Today, prions are known to cause various forms of **transmissible spongiform encephalopathy** (TSE) in human and animals. TSE is a rare degenerative disorder that affects the brain and nervous system. The accumulation of rogue proteins causes the brain tissue to become sponge-like, killing brain cells and forming holes in the tissue, leading to brain damage, loss of motor coordination, and dementia (see [\[link\]](#)). Infected individuals are mentally impaired and become unable to move or speak. There is no cure, and the disease progresses rapidly, eventually leading to death within a few months or years.



Endogenous normal prion protein (PrP^C) is converted into the disease-causing form (PrP^{Sc}) when it encounters this variant form of the protein. PrP^{Sc} may arise spontaneously in brain tissue, especially if a mutant form of the protein is present, or it may originate from misfolded prions consumed in food that eventually find their way into brain tissue. (credit b: modification of work by USDA)



Creutzfeldt-Jakob disease (CJD) is a fatal disease that causes degeneration of neural tissue. (a) These brain scans compare a normal brain to one with

CJD. (b) Compared to a normal brain, the brain tissue of a CJD patient is full of sponge-like lesions, which result from abnormal formations of prion protein. (credit a (right): modification of work by Dr. Laughlin Dawes; credit b (top): modification of work by Suzanne Wakim; credit b (bottom): modification of work by Centers for Disease Control and Prevention)

TSEs in humans include kuru, fatal familial insomnia, Gerstmann-Straussler-Scheinker disease, and Creutzfeldt-Jakob disease (see [\[link\]](#)). TSEs in animals include mad cow disease, scrapie (in sheep and goats), and chronic wasting disease (in elk and deer). TSEs can be transmitted between animals and from animals to humans by eating contaminated meat or animal feed. Transmission between humans can occur through heredity (as is often the case with GSS and CJD) or by contact with contaminated tissue, as might occur during a blood transfusion or organ transplant. There is no evidence for transmission via casual contact with an infected person. [\[link\]](#) lists TSEs that affect humans and their modes of transmission.

Transmissible Spongiform Encephalopathies (TSEs) in Humans	
Disease	Mechanism(s) of Transmission [footnote] National Institute of Neurological Disorders and Stroke. "Creutzfeldt-Jakob Disease Fact Sheet." http://www.ninds.nih.gov/disorders/cjd/detail_cjd.htm (accessed December 31, 2015).
Sporadic CJD (sCJD)	Not known; possibly by alteration of normal prior protein (PrP) to rogue form due to somatic mutation

Transmissible Spongiform Encephalopathies (TSEs) in Humans

Disease	Mechanism(s) of Transmission [footnote] National Institute of Neurological Disorders and Stroke. "Creutzfeldt-Jakob Disease Fact Sheet." http://www.ninds.nih.gov/disorders/cjd/detail_cjd.htm (accessed December 31, 2015).
Variant CJD (vCJD)	Eating contaminated cattle products and by secondary bloodborne transmission
Familial CJD (fCJD)	Mutation in germline PrP gene
Iatrogenic CJD (iCJD)	Contaminated neurosurgical instruments, corneal graft, gonadotrophic hormone, and, secondarily, by blood transfusion
Kuru	Eating infected meat through ritualistic cannibalism
Gerstmann-Straussler-Scheinker disease (GSS)	Mutation in germline PrP gene
Fatal familial insomnia (FFI)	Mutation in germline PrP gene

Prions are extremely difficult to destroy because they are resistant to heat, chemicals, and radiation. Even standard sterilization procedures do not ensure the destruction of these particles. Currently, there is no treatment or cure for TSE disease, and contaminated meats or infected animals must be handled according to federal guidelines to prevent transmission.

Note:

- Does a prion have a genome?

Note:



For more information on the handling of animals and prion-contaminated materials, visit the guidelines published on the [CDC](#) and [WHO](#) websites.

Key Concepts and Summary

- Other acellular agents such as **viroids**, **virusoids**, and **prions** also cause diseases. Viroids consist of small, naked ssRNAs that cause diseases in plants. Virusoids are ssRNAs that require other helper viruses to establish an infection. Prions are proteinaceous infectious particles that cause **transmissible spongiform encephalopathies**.
- Prions are extremely resistant to chemicals, heat, and radiation.
- There are no treatments for prion infection.

Fill in the Blank

Exercise:

Problem:

Both viroids and virusoids have a(n) _____ genome, but virusoids require a(n) _____ to reproduce.

Solution:

RNA, helper virus

Critical Thinking

Exercise:

Problem: Does a prion replicate? Explain.

Control of Microbial Growth - Introduction

class="introduction"

Most environments, including cars, are not sterile.

A study [[footnote](#)] analyzed 11 locations within 18 different cars to determine the number of microbial colony-forming units (CFUs) present. The center console harbored by far the most microbes (506 CFUs), possibly because that is where drinks are placed (and often spilled). Frequently touched sites also had high concentrations.

(credit
“photo”: modification

of work by Jeff

Wilcox)

R.E.

Stephenson et

al.

“Elucidation of
Bacteria Found
in Car Interiors
and Strategies
to Reduce the
Presence of

Potential

Pathogens.”

Biofouling 30

no. 3

(2014):337–

346.

Location	Average number CFUs per 6.5 × 6.5 cm area
Door latch	256
Door lock	14
Door lock control	182
Door handle	29
Window control	4
Cruise control button	69
Steering wheel	239
Interior steering wheel	390
Radio volume knob	99
Gear shifter	115
Center console	506



How clean is clean? People wash their cars and vacuum the carpets, but most would not want to eat from these surfaces. Similarly, we might eat with silverware cleaned in a dishwasher, but we could not use the same dishwasher to clean surgical instruments. As these examples illustrate, “clean” is a relative term. Car washing, vacuuming, and dishwashing all reduce the microbial load on the items treated, thus making them “cleaner.” But whether they are “clean enough” depends on their intended use.

Because people do not normally eat from cars or carpets, these items do not require the same level of cleanliness that silverware does. Likewise, because silverware is not used for invasive surgery, these utensils do not require the same level of cleanliness as surgical equipment, which requires sterilization to prevent infection.

Why not play it safe and sterilize everything? Sterilizing everything we come in contact with is impractical, as well as potentially dangerous. As this chapter will demonstrate, sterilization protocols often require time- and labor-intensive treatments that may degrade the quality of the item being treated or have toxic effects on users. Therefore, the user must consider the item's intended application when choosing a cleaning method to ensure that it is "clean enough."

Controlling Microbial Growth

LEARNING OBJECTIVES

- Compare disinfectants, antiseptics, and sterilants
- Describe the principles of controlling the presence of microorganisms through sterilization and disinfection
- Differentiate between microorganisms of various biological safety levels and explain methods used for handling microbes at each level

To prevent the spread of human disease, it is necessary to control the growth and abundance of microbes in or on various items frequently used by humans. Inanimate items, such as doorknobs, toys, or towels, which may harbor microbes and aid in disease transmission, are called **fomites**. Two factors heavily influence the level of cleanliness required for a particular fomite and, hence, the protocol chosen to achieve this level. The first factor is the application for which the item will be used. For example, invasive applications that require insertion into the human body require a much higher level of cleanliness than applications that do not. The second factor is the level of resistance to antimicrobial treatment by potential pathogens. For example, foods preserved by canning often become contaminated with the bacterium *Clostridium botulinum*, which produces the neurotoxin that causes botulism. Because *C. botulinum* can produce endospores that can survive harsh conditions, extreme temperatures and pressures must be used to eliminate the endospores. Other organisms may not require such extreme measures and can be controlled by a procedure such as washing clothes in a laundry machine.

Laboratory Biological Safety Levels

For researchers or laboratory personnel working with pathogens, the risks associated with specific pathogens determine the levels of cleanliness and control required. The Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) have established four classification levels, called “biological safety levels” (BSLs). Various organizations around the world, including the World Health Organization (WHO) and the European Union (EU), use a similar classification scheme. According to the CDC, the BSL is determined by the agent’s infectivity, ease of transmission, and potential disease severity, as well as the type of work being done with the agent.[\[footnote\]](#)

US Centers for Disease Control and Prevention. “Recognizing the Biosafety Levels.” <http://www.cdc.gov/training/quickearns/biosafety/>. Accessed June 7, 2016.

Each BSL requires a different level of biocontainment to prevent contamination and spread of infectious agents to laboratory personnel and, ultimately, the community. For example, the lowest BSL, BSL-1, requires the fewest precautions because it applies to situations with the lowest risk for microbial infection.

BSL-1 agents are those that generally do not cause infection in healthy human adults. These include noninfectious bacteria, such as nonpathogenic strains of *Escherichia coli* and *Bacillus subtilis*, and viruses known to infect animals other than humans, such as baculoviruses (insect viruses). Because working with BSL-1 agents poses very little risk, few precautions are necessary. Laboratory workers use standard aseptic technique and may work with these agents at an open laboratory bench or table, wearing personal protective equipment (PPE) such as a laboratory coat, goggles, and gloves, as needed. Other than a sink for handwashing and doors to separate the laboratory from the rest of the building, no additional modifications are needed.

Agents classified as BSL-2 include those that pose moderate risk to laboratory workers and the community, and are typically “indigenous,” meaning that they are commonly found in that geographical area. These

include bacteria such as *Staphylococcus aureus* and *Salmonella* spp., and viruses like hepatitis, mumps, and measles viruses. BSL-2 laboratories require additional precautions beyond those of BSL-1, including restricted access; required PPE, including a face shield in some circumstances; and the use of biological safety cabinets for procedures that may disperse agents through the air (called “aerosolization”). BSL-2 laboratories are equipped with self-closing doors, an eyewash station, and an **autoclave**, which is a specialized device for sterilizing materials with pressurized steam before use or disposal. BSL-1 laboratories may also have an autoclave.

BSL-3 agents have the potential to cause lethal infections by inhalation. These may be either indigenous or “exotic,” meaning that they are derived from a foreign location, and include pathogens such as *Mycobacterium tuberculosis*, *Bacillus anthracis*, West Nile virus, and human immunodeficiency virus (HIV). Because of the serious nature of the infections caused by BSL-3 agents, laboratories working with them require restricted access. Laboratory workers are under medical surveillance, possibly receiving vaccinations for the microbes with which they work. In addition to the standard PPE already mentioned, laboratory personnel in BSL-3 laboratories must also wear a respirator and work with microbes and infectious agents in a biological safety cabinet at all times. BSL-3 laboratories require a hands-free sink, an eyewash station near the exit, and two sets of self-closing and locking doors at the entrance. These laboratories are equipped with directional airflow, meaning that clean air is pulled through the laboratory from clean areas to potentially contaminated areas. This air cannot be recirculated, so a constant supply of clean air is required.

BSL-4 agents are the most dangerous and often fatal. These microbes are typically exotic, are easily transmitted by inhalation, and cause infections for which there are no treatments or vaccinations. Examples include Ebola virus and Marburg virus, both of which cause hemorrhagic fevers, and smallpox virus. There are only a small number of laboratories in the United States and around the world appropriately equipped to work with these agents. In addition to BSL-3 precautions, laboratory workers in BSL-4 facilities must also change their clothing on entering the laboratory, shower on exiting, and decontaminate all material on exiting. While working in the

laboratory, they must either wear a full-body protective suit with a designated air supply or conduct all work within a biological safety cabinet with a high-efficiency particulate air (HEPA)-filtered air supply and a doubly HEPA-filtered exhaust. If wearing a suit, the air pressure within the suit must be higher than that outside the suit, so that if a leak in the suit occurs, laboratory air that may be contaminated cannot be drawn into the suit ([\[link\]](#)). The laboratory itself must be located either in a separate building or in an isolated portion of a building and have its own air supply and exhaust system, as well as its own decontamination system. The BSUs are summarized in [\[link\]](#).



A protective suit like this one is an additional precaution for those who work in BSL-4 laboratories. This suit has its own air supply and maintains a positive pressure relative to the outside, so that if a leak occurs, air will flow out of the suit, not into it from the laboratory.
(credit: modification of work by Centers for Disease Control and Prevention)

Biosafety Levels			
Biological Safety Levels	Description	Examples	CDC Classification
BSL-4	Microbes are dangerous and exotic, posing a high risk of aerosol-transmitted infections, which are frequently fatal without treatment or vaccines. Few labs are at this level.	Ebola and Marburg viruses	
BSL-3	Microbes are indigenous or exotic and cause serious or potentially lethal diseases through respiratory transmission.	<i>Mycobacterium tuberculosis</i>	
BSL-2	Microbes are typically indigenous and are associated with diseases of varying severity. They pose moderate risk to workers and the environment.	<i>Staphylococcus aureus</i>	
BSL-1	Microbes are not known to cause disease in healthy hosts and pose minimal risk to workers and the environment.	Nonpathogenic strains of <i>Escherichia coli</i>	<p>BSL-4</p> <p>BSL-3</p> <p>BSL-2</p> <p>BSL-1</p> <p>high-risk microbes</p> <p>low-risk microbes</p>

The CDC classifies infectious agents into four biosafety levels based on potential risk to laboratory personnel and the community. Each level requires a progressively greater level of precaution. (credit “pyramid”: modification of work by Centers for Disease Control and Prevention)

Note:



To [learn more](#) about the four BSls, visit the CDC's website.

Note:

- What are some factors used to determine the BSL necessary for working with a specific pathogen?

Sterilization

The most extreme protocols for microbial control aim to achieve **sterilization**: the complete removal or killing of all vegetative cells, endospores, and viruses from the targeted item or environment. Sterilization protocols are generally reserved for laboratory, medical, manufacturing, and food industry settings, where it may be imperative for certain items to be completely free of potentially infectious agents. Sterilization can be accomplished through either physical means, such as exposure to high heat, pressure, or filtration through an appropriate filter, or by chemical means. Chemicals that can be used to achieve sterilization are called **sterilants**. Sterilants effectively kill all microbes and viruses, and, with appropriate exposure time, can also kill endospores.

For many clinical purposes, **aseptic technique** is necessary to prevent contamination of sterile surfaces. Aseptic technique involves a combination of protocols that collectively maintain sterility, or **asepsis**, thus preventing contamination of the patient with microbes and infectious agents. Failure to

practice aseptic technique during many types of clinical procedures may introduce microbes to the patient's body and put the patient at risk for **sepsis**, a systemic inflammatory response to an infection that results in high fever, increased heart and respiratory rates, shock, and, possibly, death. Medical procedures that carry risk of contamination must be performed in a **sterile field**, a designated area that is kept free of all vegetative microbes, endospores, and viruses. Sterile fields are created according to protocols requiring the use of sterilized materials, such as packaging and drapings, and strict procedures for washing and application of sterilants. Other protocols are followed to maintain the sterile field while the medical procedure is being performed.

One food sterilization protocol, **commercial sterilization**, uses heat at a temperature low enough to preserve food quality but high enough to destroy common pathogens responsible for food poisoning, such as *C. botulinum*. Because *C. botulinum* and its endospores are commonly found in soil, they may easily contaminate crops during harvesting, and these endospores can later germinate within the anaerobic environment once foods are canned. Metal cans of food contaminated with *C. botulinum* will bulge due to the microbe's production of gases; contaminated jars of food typically bulge at the metal lid. To eliminate the risk for *C. botulinum* contamination, commercial food-canning protocols are designed with a large margin of error. They assume an impossibly large population of endospores (10^{12} per can) and aim to reduce this population to 1 endospore per can to ensure the safety of canned foods. For example, low- and medium-acid foods are heated to 121 °C for a minimum of 2.52 minutes, which is the time it would take to reduce a population of 10^{12} endospores per can down to 1 endospore at this temperature. Even so, commercial sterilization does not eliminate the presence of all microbes; rather, it targets those pathogens that cause spoilage and foodborne diseases, while allowing many nonpathogenic organisms to survive. Therefore, "sterilization" is somewhat of a misnomer in this context, and commercial sterilization may be more accurately described as "quasi-sterilization."

Note:

- What is the difference between sterilization and aseptic technique?

Note:



The Association of Surgical Technologists publishes [standards](#) for aseptic technique, including creating and maintaining a sterile field.

Other Methods of Control

Sterilization protocols require procedures that are not practical, or necessary, in many settings. Various other methods are used in clinical and nonclinical settings to reduce the microbial load on items. Although the terms for these methods are often used interchangeably, there are important distinctions ([\[link\]](#)).

The process of **disinfection** inactivates most microbes on the surface of a fomite by using antimicrobial chemicals or heat. Because some microbes remain, the disinfected item is not considered sterile. Ideally, **disinfectants** should be fast acting, stable, easy to prepare, inexpensive, and easy to use. An example of a natural disinfectant is vinegar; its acidity kills most microbes. Chemical disinfectants, such as chlorine bleach or products containing chlorine, are used to clean nonliving surfaces such as laboratory benches, clinical surfaces, and bathroom sinks. Typical disinfection does not lead to sterilization because endospores tend to survive even when all vegetative cells have been killed.

Unlike disinfectants, **antiseptics** are antimicrobial chemicals safe for use on living skin or tissues. Examples of antiseptics include hydrogen peroxide and isopropyl alcohol. The process of applying an antiseptic is called **antisepsis**. In addition to the characteristics of a good disinfectant, antiseptics must also be selectively effective against microorganisms and able to penetrate tissue deeply without causing tissue damage.

The type of protocol required to achieve the desired level of cleanliness depends on the particular item to be cleaned. For example, those used clinically are categorized as critical, semicritical, and noncritical. Critical items must be sterile because they will be used inside the body, often penetrating sterile tissues or the bloodstream; examples of **critical items** include surgical instruments, catheters, and intravenous fluids. Gastrointestinal endoscopes and various types of equipment for respiratory therapies are examples of **semicritical items**; they may contact mucous membranes or nonintact skin but do not penetrate tissues. Semicritical items do not typically need to be sterilized but do require a high level of disinfection. Items that may contact but not penetrate intact skin are **noncritical items**; examples are bed linens, furniture, crutches, stethoscopes, and blood pressure cuffs. These articles need to be clean but not highly disinfected.

The act of handwashing is an example of **degerming**, in which microbial numbers are significantly reduced by gently scrubbing living tissue, most commonly skin, with a mild chemical (e.g., soap) to avoid the transmission of pathogenic microbes. Wiping the skin with an alcohol swab at an injection site is another example of degerning. These degerning methods remove most (but not all) microbes from the skin's surface.

The term **sanitization** refers to the cleansing of fomites to remove enough microbes to achieve levels deemed safe for public health. For example, commercial dishwashers used in the food service industry typically use very hot water and air for washing and drying; the high temperatures kill most microbes, sanitizing the dishes. Surfaces in hospital rooms are commonly sanitized using a chemical disinfectant to prevent disease transmission between patients. [\[link\]](#) summarizes common protocols, definitions, applications, and agents used to control microbial growth.

Common Protocols for Control of Microbial Growth			
Protocol	Definition	Common Application	Common Agents
For Use on Fomites			
Disinfection	Reduces or destroys microbial load of an inanimate item through application of heat or antimicrobial chemicals	Cleaning surfaces like laboratory benches, clinical surfaces, and bathrooms	Chlorine bleach, phenols (e.g., Lysol), glutaraldehyde
Sanitization	Reduces microbial load of an inanimate item to safe public health levels through application of heat or antimicrobial chemicals	Commercial dishwashing of eating utensils, cleaning public restrooms	Detergents containing phosphates (e.g., Finish), industrial-strength cleaners containing quaternary ammonium compounds
Sterilization	Completely eliminates all vegetative cells, endospores, and viruses from an inanimate item	Preparation of surgical equipment and of needles used for injection	Pressurized steam (autoclave), chemicals, radiation
For Use on Living Tissue			
Antisepsis	Reduces microbial load on skin or tissue through application of an antimicrobial chemical	Cleaning skin broken due to injury; cleaning skin before surgery	Boric acid, isopropyl alcohol, hydrogen peroxide, iodine (betadine)
Degerming	Reduces microbial load on skin or tissue through gentle to firm scrubbing and the use of mild chemicals	Handwashing	Soap, alcohol swab

Note:

- What is the difference between a disinfectant and an antiseptic?
- Which is most effective at removing microbes from a product: sanitization, degerning, or sterilization? Explain.

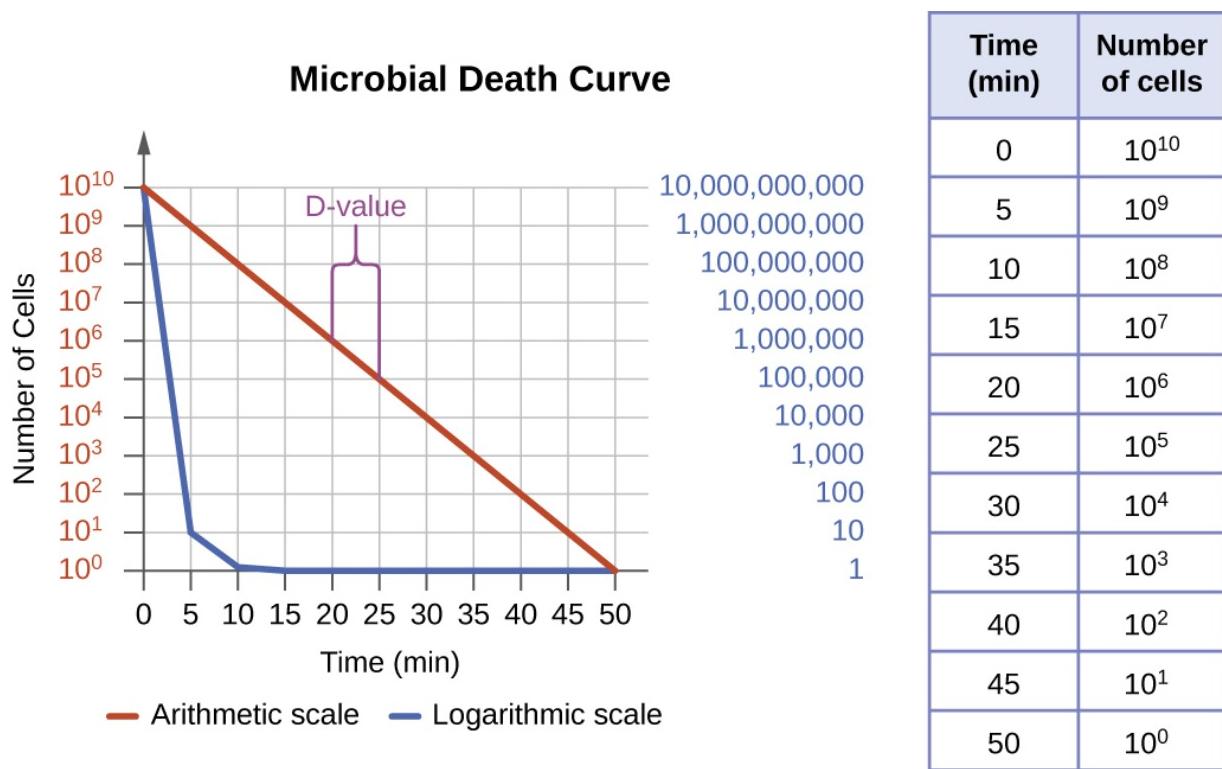
Measuring Microbial Control

Physical and chemical methods of microbial control that kill the targeted microorganism are identified by the suffix *-cide* (or *-cidal*). The prefix indicates the type of microbe or infectious agent killed by the treatment method: **bactericides** kill bacteria, **viricides** kill or inactivate viruses, and **fungicides** kill fungi. Other methods do not kill organisms but, instead, stop

their growth, making their population static; such methods are identified by the suffix *-stat* (or *-static*). For example, **bacteriostatic** treatments inhibit the growth of bacteria, whereas **fungistatic** treatments inhibit the growth of fungi. Factors that determine whether a particular treatment is *-cidal* or *-static* include the types of microorganisms targeted, the concentration of the chemical used, and the nature of the treatment applied.

Although *-static* treatments do not actually kill infectious agents, they are often less toxic to humans and other animals, and may also better preserve the integrity of the item treated. Such treatments are typically sufficient to keep the microbial population of an item in check. The reduced toxicity of some of these *-static* chemicals also allows them to be impregnated safely into plastics to prevent the growth of microbes on these surfaces. Such plastics are used in products such as toys for children and cutting boards for food preparation. When used to treat an infection, *-static* treatments are typically sufficient in an otherwise healthy individual, preventing the pathogen from multiplying, thus allowing the individual's immune system to clear the infection.

The degree of microbial control can be evaluated using a **microbial death curve** to describe the progress and effectiveness of a particular protocol. When exposed to a particular microbial control protocol, a fixed percentage of the microbes within the population will die. Because the rate of killing remains constant even when the population size varies, the percentage killed is more useful information than the absolute number of microbes killed. Death curves are often plotted as semilog plots just like microbial growth curves because the reduction in microorganisms is typically logarithmic ([\[link\]](#)). The amount of time it takes for a specific protocol to produce a one order-of-magnitude decrease in the number of organisms, or the death of 90% of the population, is called the **decimal reduction time (DRT) or D-value**.



Microbial death is logarithmic and easily observed using a semilog plot instead of an arithmetic one. The decimal reduction time (D-value) is the time it takes to kill 90% of the population (a 1-log decrease in the total population) when exposed to a specific microbial control protocol, as indicated by the purple bracket.

Several factors contribute to the effectiveness of a disinfecting agent or microbial control protocol. First, as demonstrated in [\[link\]](#), the length of time of exposure is important. Longer exposure times kill more microbes. Because microbial death of a population exposed to a specific protocol is logarithmic, it takes longer to kill a high-population load than a low-population load exposed to the same protocol. A shorter treatment time (measured in multiples of the D-value) is needed when starting with a smaller number of organisms. Effectiveness also depends on the susceptibility of the agent to that disinfecting agent or protocol. The concentration of disinfecting agent or intensity of exposure is also important. For example, higher temperatures and higher concentrations of

disinfectants kill microbes more quickly and effectively. Conditions that limit contact between the agent and the targeted cells—cells—for example, the presence of bodily fluids, tissue, organic debris (e.g., mud or feces), or biofilms on surfaces—increase the cleaning time or intensity of the microbial control protocol required to reach the desired level of cleanliness. All these factors must be considered when choosing the appropriate protocol to control microbial growth in a given situation.

Note:

- What are two possible reasons for choosing a bacteriostatic treatment over a bactericidal one?
- Name at least two factors that can compromise the effectiveness of a disinfecting agent.

Key Concepts and Summary

- Inanimate items that may harbor microbes and aid in their transmission are called **fomites**. The level of cleanliness required for a fomite depends both on the item’s use and the infectious agent with which the item may be contaminated.
- The CDC and the NIH have established four **biological safety levels (BSLs)** for laboratories performing research on infectious agents. Each level is designed to protect laboratory personnel and the community. These BSLS are determined by the agent’s infectivity, ease of transmission, and potential disease severity, as well as the type of work being performed with the agent.
- **Disinfection** removes potential pathogens from a fomite, whereas **antisepsis** uses antimicrobial chemicals safe enough for tissues; in both cases, microbial load is reduced, but microbes may remain unless the chemical used is strong enough to be a **sterilant**.
- The amount of cleanliness (**sterilization** versus high-level disinfection versus general cleanliness) required for items used clinically depends

on whether the item will come into contact with sterile tissues (**critical item**), mucous membranes (**semicritical item**), or intact skin (**noncritical item**).

- Medical procedures with a risk for contamination should be carried out in a **sterile field** maintained by proper **aseptic technique** to prevent **sepsis**.
- Sterilization is necessary for some medical applications as well as in the food industry, where endospores of *Clostridium botulinum* are killed through **commercial sterilization** protocols.
- Physical or chemical methods to control microbial growth that result in death of the microbe are indicated by the suffixes *-cide* or *-cidal* (e.g., as with **bactericides**, **viricides**, and **fungicides**), whereas those that inhibit microbial growth are indicated by the suffixes *-stat* or *-static* (e.g., **bacteriostatic**, **fungistatic**).
- **Microbial death curves** display the logarithmic decline of living microbes exposed to a method of microbial control. The time it takes for a protocol to yield a 1-log (90%) reduction in the microbial population is the **decimal reduction time**, or **D-value**.
- When choosing a microbial control protocol, factors to consider include the length of exposure time, the type of microbe targeted, its susceptibility to the protocol, the intensity of the treatment, the presence of organics that may interfere with the protocol, and the environmental conditions that may alter the effectiveness of the protocol.

Short Answer

Exercise:

Problem:

What are some characteristics of microbes and infectious agents that would require handling in a BSL-3 laboratory?

Exercise:

Problem:

What is the purpose of degerming? Does it completely eliminate microbes?

Exercise:**Problem:**

What are some factors that alter the effectiveness of a disinfectant?

Critical Thinking

Exercise:**Problem:**

When plotting microbial death curves, how might they look different for bactericidal versus bacteriostatic treatments?

Exercise:**Problem:**

What are the benefits of cleaning something to a level of cleanliness beyond what is required? What are some possible disadvantages of doing so?

Using Physical Methods to Control Microorganisms

LEARNING OBJECTIVES

- Understand and compare various physical methods of controlling microbial growth, including heating, refrigeration, freezing, high-pressure treatment, desiccation, lyophilization, irradiation, and filtration

For thousands of years, humans have used various physical methods of microbial control for food preservation. Common control methods include the application of high temperatures, radiation, filtration, and desiccation (drying), among others. Many of these methods nonspecifically kill cells by disrupting membranes, changing membrane permeability, or damaging proteins and nucleic acids by denaturation, degradation, or chemical modification. Various physical methods used for microbial control are described in this section.

Heat

Heating is one of the most common—and oldest—forms of microbial control. It is used in simple techniques like cooking and canning. Heat can kill microbes by altering their membranes and denaturing proteins. The **thermal death point (TDP)** of a microorganism is the lowest temperature at which all microbes are killed in a 10-minute exposure. Different microorganisms will respond differently to high temperatures, with some (e.g., endospore-formers such as *C. botulinum*) being more heat tolerant. A similar parameter, the **thermal death time (TDT)**, is the length of time needed to kill all microorganisms in a sample at a given temperature. These parameters are often used to describe sterilization procedures that use high heat, such as autoclaving. Boiling is one of the oldest methods of moist-heat control of

microbes, and it is typically quite effective at killing vegetative cells and some viruses. However, boiling is less effective at killing endospores; some endospores are able to survive up to 20 hours of boiling. Additionally, boiling may be less effective at higher altitudes, where the boiling point of water is lower and the boiling time needed to kill microbes is therefore longer. For these reasons, boiling is not considered a useful sterilization technique in the laboratory or clinical setting.

Many different heating protocols can be used for sterilization in the laboratory or clinic, and these protocols can be broken down into two main categories: **dry-heat sterilization** and **moist-heat sterilization**. Aseptic technique in the laboratory typically involves some dry-heat sterilization protocols using direct application of high heat, such as sterilizing inoculating loops ([\[link\]](#)). Incineration at very high temperatures destroys all microorganisms. Dry heat can also be applied for relatively long periods of time (at least 2 hours) at temperatures up to 170 °C by using a dry-heat sterilizer, such as an oven. However, moist-heat sterilization is typically the more effective protocol because it penetrates cells better than dry heat does.



(a)



(b)

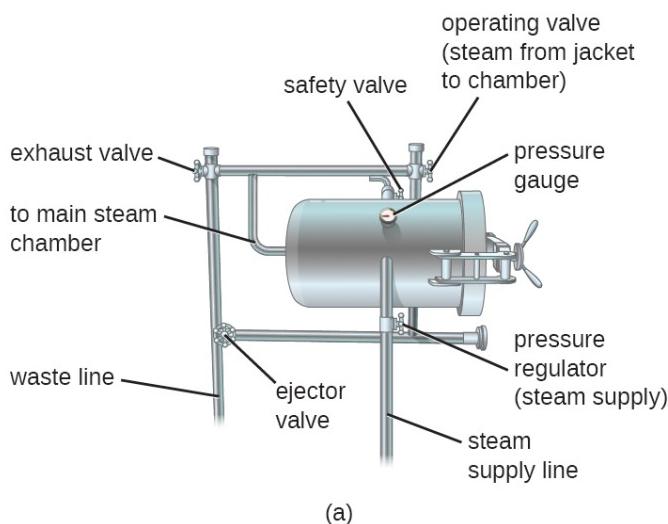
(a) Sterilizing a loop, often referred to as “flaming a loop,” is a common component of aseptic technique in the microbiology laboratory and is used to incinerate any microorganisms on the loop. (b) Alternatively, a bactericinerator may be used to reduce aerosolization of microbes and remove the presence of an open flame in the laboratory. These are examples of dry-heat sterilization by the direct application of high heat capable of incineration. (credit a: modification of work by Anh-Hue Tu; credit b: modification of work by Brian Forster)

Autoclaves

Autoclaves rely on moist-heat sterilization. They are used to raise temperatures above the boiling point of water to sterilize items such as surgical equipment from vegetative cells, viruses, and especially endospores, which are known to survive boiling temperatures, without damaging the items. Charles

Chamberland (1851–1908) designed the modern autoclave in 1879 while working in the laboratory of Louis Pasteur. The autoclave is still considered the most effective method of sterilization ([\[link\]](#)). Outside laboratory and clinical settings, large industrial autoclaves called **retorts** allow for moist-heat sterilization on a large scale.

In general, the air in the chamber of an autoclave is removed and replaced with increasing amounts of steam trapped within the enclosed chamber, resulting in increased interior pressure and temperatures above the boiling point of water. The two main types of autoclaves differ in the way that air is removed from the chamber. In gravity displacement autoclaves, steam is introduced into the chamber from the top or sides. Air, which is heavier than steam, sinks to the bottom of the chamber, where it is forced out through a vent. Complete displacement of air is difficult, especially in larger loads, so longer cycles may be required for such loads. In prevacuum sterilizers, air is removed completely using a high-speed vacuum before introducing steam into the chamber. Because air is more completely eliminated, the steam can more easily penetrate wrapped items. Many autoclaves are capable of both gravity and prevacuum cycles, using the former for the decontamination of waste and sterilization of media and unwrapped glassware, and the latter for sterilization of packaged instruments.



(a)



(b)

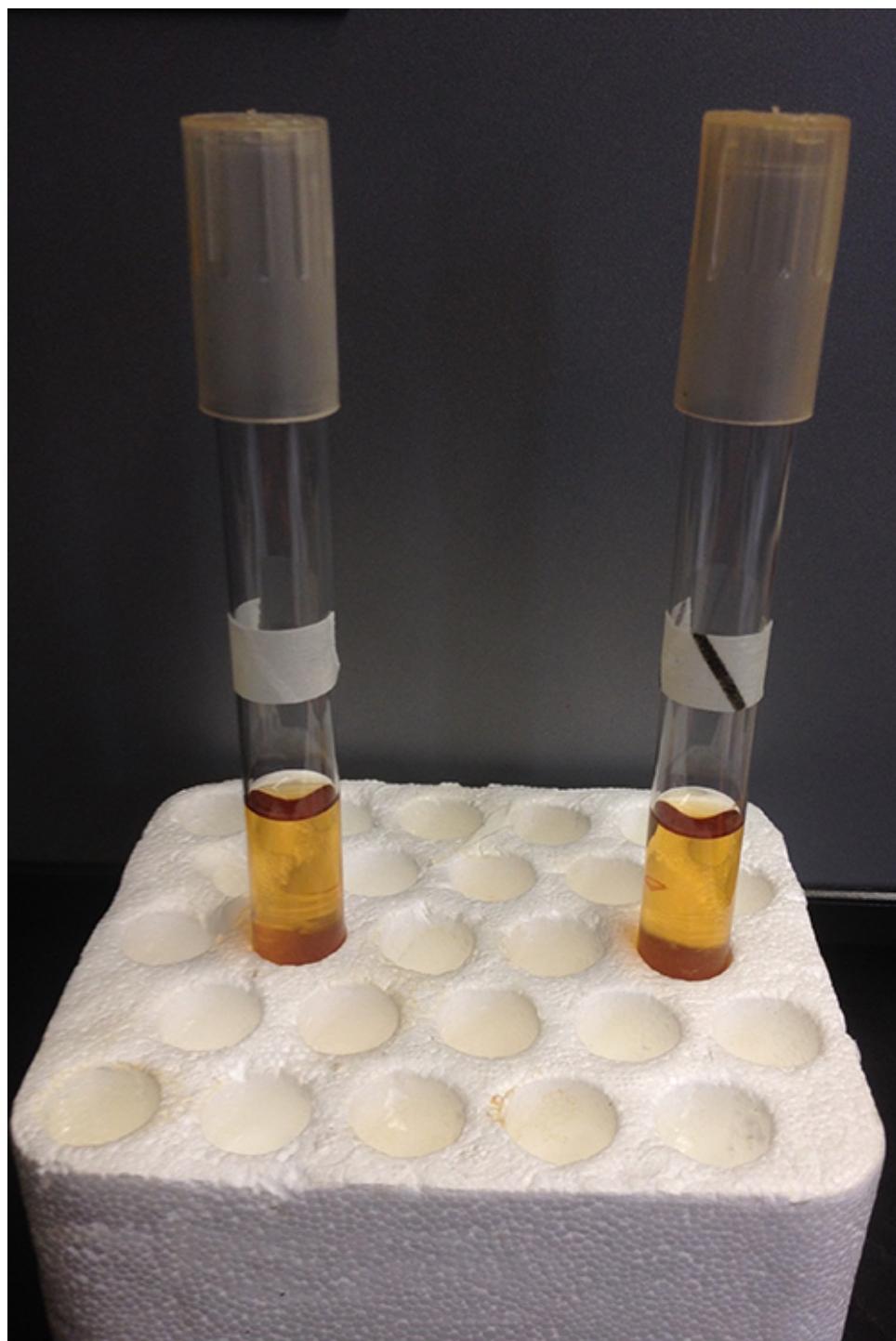
- (a) An autoclave is commonly used for sterilization in the laboratory and in clinical settings. By displacing the air in the chamber with increasing

amounts of steam, pressure increases, and temperatures exceeding 100 °C can be achieved, allowing for complete sterilization. (b) A researcher programs an autoclave to sterilize a sample. (credit a: modification of work by Courtney Harrington; credit b: modification of work by Lackemeyer MG, Kok-Mercado Fd, Wada J, Bollinger L, Kindrachuk J, Wahl-Jensen V, Kuhn JH, Jahrling PB)

Standard operating temperatures for autoclaves are 121 °C or, in some cases, 132 °C, typically at a pressure of 15 to 20 pounds per square inch (psi). The length of exposure depends on the volume and nature of material being sterilized, but it is typically 20 minutes or more, with larger volumes requiring longer exposure times to ensure sufficient heat transfer to the materials being sterilized. The steam must directly contact the liquids or dry materials being sterilized, so containers are left loosely closed and instruments are loosely wrapped in paper or foil. The key to autoclaving is that the temperature must be high enough to kill endospores to achieve complete sterilization.

Because sterilization is so important to safe medical and laboratory protocols, quality control is essential. Autoclaves may be equipped with recorders to document the pressures and temperatures achieved during each run. Additionally, internal indicators of various types should be autoclaved along with the materials to be sterilized to ensure that the proper sterilization temperature has been reached ([\[link\]](#)). One common type of indicator is the use of heat-sensitive autoclave tape, which has white stripes that turn black when the appropriate temperature is achieved during a successful autoclave run. This type of indicator is relatively inexpensive and can be used during every run. However, autoclave tape provides no indication of length of exposure, so it cannot be used as an indicator of sterility. Another type of indicator, a biological indicator spore test, uses either a strip of paper or a liquid suspension of the endospores of *Geobacillus stearothermophilus* to determine whether the endospores are killed by the process. The endospores of the obligate thermophilic bacterium *G. stearothermophilus* are the gold standard used for this purpose because of their extreme heat resistance. Biological spore indicators can also be used to test the effectiveness of other sterilization protocols, including ethylene oxide, dry heat, formaldehyde, gamma radiation, and hydrogen peroxide plasma sterilization using either *G. stearothermophilus*, *Bacillus atrophaeus*, *B. subtilis*, or *B. pumilus* spores. In

the case of validating autoclave function, the endospores are incubated after autoclaving to ensure no viable endospores remain. Bacterial growth subsequent to endospore germination can be monitored by biological indicator spore tests that detect acid metabolites or fluorescence produced by enzymes derived from viable *G. stearothermophilus*. A third type of autoclave indicator is the Diack tube, a glass ampule containing a temperature-sensitive pellet that melts at the proper sterilization temperature. Spore strips or Diack tubes are used periodically to ensure the autoclave is functioning properly.

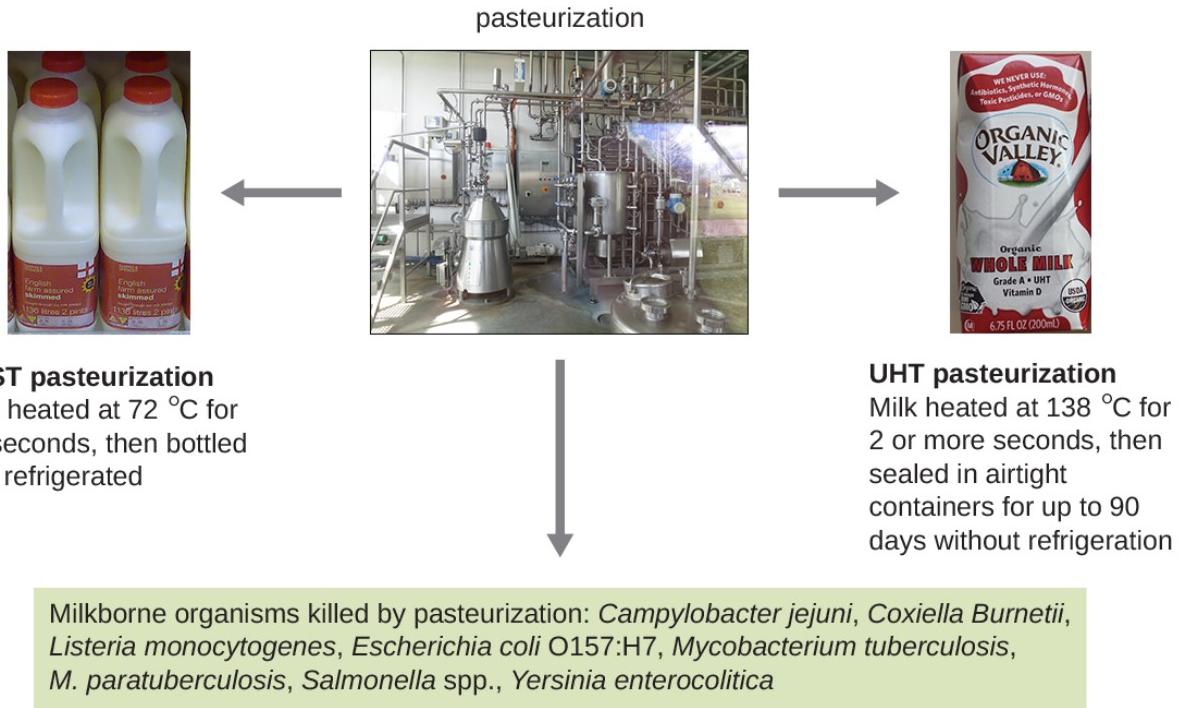


The white strips on autoclave tape (left tube) turn dark during a successful autoclave run (right tube). (credit: modification of work by Brian Forster)

Pasteurization

Although complete sterilization is ideal for many medical applications, it is not always practical for other applications and may also alter the quality of the product. Boiling and autoclaving are not ideal ways to control microbial growth in many foods because these methods may ruin the consistency and other organoleptic (sensory) qualities of the food. Pasteurization is a form of microbial control for food that uses heat but does not render the food sterile. Traditional **pasteurization** kills pathogens and reduces the number of spoilage-causing microbes while maintaining food quality. The process of pasteurization was first developed by Louis Pasteur in the 1860s as a method for preventing the spoilage of beer and wine. Today, pasteurization is most commonly used to kill heat-sensitive pathogens in milk and other food products (e.g., apple juice and honey) ([\[link\]](#)). However, because pasteurized food products are not sterile, they will eventually spoil.

The methods used for milk pasteurization balance the temperature and the length of time of treatment. One method, **high-temperature short-time (HTST) pasteurization**, exposes milk to a temperature of 72 °C for 15 seconds, which lowers bacterial numbers while preserving the quality of the milk. An alternative is **ultra-high-temperature (UHT) pasteurization**, in which the milk is exposed to a temperature of 138 °C for 2 or more seconds. UHT pasteurized milk can be stored for a long time in sealed containers without being refrigerated; however, the very high temperatures alter the proteins in the milk, causing slight changes in the taste and smell. Still, this method of pasteurization is advantageous in regions where access to refrigeration is limited.



Two different methods of pasteurization, HTST and UHT, are commonly used to kill pathogens associated with milk spoilage. (credit left: modification of work by Mark Hillary; credit right: modification of work by Kerry Ceszyk)

Note:

- In an autoclave, how are temperatures above boiling achieved?
- How would the onset of spoilage compare between HTST-pasteurized and UHT-pasteurized milk?
- Why is boiling not used as a sterilization method in a clinical setting?

Refrigeration and Freezing

Just as high temperatures are effective for controlling microbial growth, exposing microbes to low temperatures can also be an easy and effective method of microbial control, with the exception of psychrophiles, which prefer cold temperatures (see [Temperature and Microbial Growth](#)). Refrigerators used in home kitchens or in the laboratory maintain temperatures between 0 °C and 7 °C. This temperature range inhibits microbial metabolism, slowing the growth of microorganisms significantly and helping preserve refrigerated products such as foods or medical supplies. Certain types of laboratory cultures can be preserved by refrigeration for later use.

Freezing below –2 °C may stop microbial growth and even kill susceptible organisms. According to the US Department of Agriculture (USDA), the only safe ways that frozen foods can be thawed are in the refrigerator, immersed in cold water changed every 30 minutes, or in the microwave, keeping the food at temperatures not conducive for bacterial growth.[\[footnote\]](#) In addition, halted bacterial growth can restart in thawed foods, so thawed foods should be treated like fresh perishables.

US Department of Agriculture. “Freezing and Food Safety.” 2013. http://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/safe-food-handling/freezing-and-food-safety/CT_Index. Accessed June 8, 2016.

Bacterial cultures and medical specimens requiring long-term storage or transport are often frozen at ultra-low temperatures of –70 °C or lower. These ultra-low temperatures can be achieved by storing specimens on dry ice in an ultra-low freezer or in special liquid nitrogen tanks, which maintain temperatures lower than –196 °C ([\[link\]](#)).



(a)



(b)

Cultures and other medical specimens can be stored for long periods at ultra-low temperatures. (a) An ultra-low freezer maintains temperatures at or below -70°C . (b) Even lower temperatures can be achieved through freezing and storage in liquid nitrogen. (credit a: modification of work by “Expert Infantry”/Flickr; credit b: modification of work by USDA)

Note:

- Does placing food in a refrigerator kill bacteria on the food?

Pressure

Exposure to high pressure kills many microbes. In the food industry, high-pressure processing (also called pascalization) is used to kill bacteria, yeast, molds, parasites, and viruses in foods while maintaining food quality and extending shelf life. The application of high pressure between 100 and 800

MPa (sea level atmospheric pressure is about 0.1 MPa) is sufficient to kill vegetative cells by protein denaturation, but endospores may survive these pressures.[\[footnote\]](#)[\[footnote\]](#)

C. Ferstl. "High Pressure Processing: Insights on Technology and Regulatory Requirements." Food for Thought/White Paper. Series Volume 10. Livermore, CA: The National Food Lab; July 2013.

US Food and Drug Administration. "Kinetics of Microbial Inactivation for Alternative Food Processing Technologies: High Pressure Processing." 2000. <http://www.fda.gov/Food/FoodScienceResearch/SafePracticesforFoodProcesses/ucm101456.htm>. Accessed July 19, 2106.

In clinical settings, hyperbaric oxygen therapy is sometimes used to treat infections. In this form of therapy, a patient breathes pure oxygen at a pressure higher than normal atmospheric pressure, typically between 1 and 3 atmospheres (atm). This is achieved by placing the patient in a hyperbaric chamber or by supplying the pressurized oxygen through a breathing tube. Hyperbaric oxygen therapy helps increase oxygen saturation in tissues that become hypoxic due to infection and inflammation. This increased oxygen concentration enhances the body's immune response by increasing the activities of neutrophils and macrophages, white blood cells that fight infections. Increased oxygen levels also contribute to the formation of toxic free radicals that inhibit the growth of oxygen-sensitive or anaerobic bacteria like as *Clostridium perfringens*, a common cause of gas gangrene. In *C. perfringens* infections, hyperbaric oxygen therapy can also reduce secretion of a bacterial toxin that causes tissue destruction. Hyperbaric oxygen therapy also seems to enhance the effectiveness of antibiotic treatments. Unfortunately, some rare risks include oxygen toxicity and effects on delicate tissues, such as the eyes, middle ear, and lungs, which may be damaged by the increased air pressure.

High pressure processing is not commonly used for disinfection or sterilization of fomites. Although the application of pressure and steam in an autoclave is effective for killing endospores, it is the high temperature achieved, and not the pressure directly, that results in endospore death.

Note:



To [learn more](#) about proper home-canning techniques, visit the CDC's website.

Desiccation

Drying, also known as **desiccation** or dehydration, is a method that has been used for millennia to preserve foods such as raisins, prunes, and jerky. It works because all cells, including microbes, require water for their metabolism and survival. Although drying controls microbial growth, it might not kill all microbes or their endospores, which may start to regrow when conditions are more favorable and water content is restored.

In some cases, foods are dried in the sun, relying on evaporation to achieve desiccation. Freeze-drying, or **lyophilization**, is another method of dessication in which an item is rapidly frozen (“snap-frozen”) and placed under vacuum so that water is lost by sublimation. Lyophilization combines both exposure to cold temperatures and desiccation, making it quite effective for controlling microbial growth. In addition, lyophilization causes less damage to an item than conventional desiccation and better preserves the item’s original qualities. Lyophilized items may be stored at room temperature if packaged appropriately to prevent moisture acquisition. Lyophilization is used for preservation in the food industry and is also used in the laboratory for the long-term storage and transportation of microbial cultures.

The water content of foods and materials, called the **water activity**, can be lowered without physical drying by the addition of solutes such as salts or sugars. At very high concentrations of salts or sugars, the amount of available water in microbial cells is reduced dramatically because water will be drawn from an area of low solute concentration (inside the cell) to an area of high

solute concentration (outside the cell) ([\[link\]](#)). Many microorganisms do not survive these conditions of high osmotic pressure. Honey, for example, is 80% sucrose, an environment in which very few microorganisms are capable of growing, thereby eliminating the need for refrigeration. Salted meats and fish, like ham and cod, respectively, were critically important foods before the age of refrigeration. Fruits were preserved by adding sugar, making jams and jellies. However, certain microbes, such as molds and yeasts, tend to be more tolerant of desiccation and high osmotic pressures, and, thus, may still contaminate these types of foods.



(a) The addition of a solute creates a hypertonic environment, drawing water out of cells. (b) Some foods can be dried directly, like raisins and jerky. Other foods are dried with the addition of salt, as in the case of salted fish, or sugar, as in the case of jam. (credit a: modification of work by “Bruce Blaus”/Wikimedia Commons; credit raisins: modification of work by Christian Schnettelker; credit jerky: modification of work by Larry Jacobsen; credit salted fish: modification of work by “The Photographer”/Wikimedia Commons; credit jam: modification of work by Kim Becker)

Note:

- How does the addition of salt or sugar to food affect its water activity?

Radiation

Radiation in various forms, from high-energy radiation to sunlight, can be used to kill microbes or inhibit their growth. **Ionizing radiation** includes X-rays, gamma rays, and high-energy electron beams. Ionizing radiation is strong enough to pass into the cell, where it alters molecular structures and damages cell components. For example, ionizing radiation introduces double-strand breaks in DNA molecules. This may directly cause DNA mutations to occur, or mutations may be introduced when the cell attempts to repair the DNA damage. As these mutations accumulate, they eventually lead to cell death.

Both X-rays and gamma rays easily penetrate paper and plastic and can therefore be used to sterilize many packaged materials. In the laboratory, ionizing radiation is commonly used to sterilize materials that cannot be autoclaved, such as plastic Petri dishes and disposable plastic inoculating loops. For clinical use, ionizing radiation is used to sterilize gloves, intravenous tubing, and other latex and plastic items used for patient care. Ionizing radiation is also used for the sterilization of other types of delicate, heat-sensitive materials used clinically, including tissues for transplantation, pharmaceutical drugs, and medical equipment.

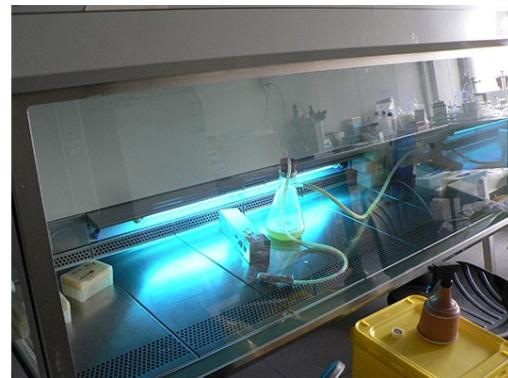
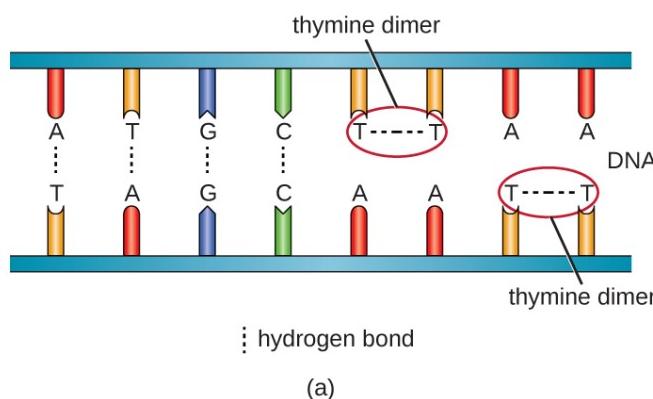
In Europe, gamma irradiation for food preservation is widely used, although it has been slow to catch on in the United States (see the [Micro Connections](#) box on this topic). Packaged dried spices are also often gamma-irradiated. Because of their ability to penetrate paper, plastic, thin sheets of wood and metal, and tissue, great care must be taken when using X-rays and gamma irradiation. These types of ionizing irradiation cannot penetrate thick layers of iron or lead, so these metals are commonly used to protect humans who may be potentially exposed.

Another type of radiation, **nonionizing radiation**, is commonly used for sterilization and uses less energy than ionizing radiation. It does not penetrate cells or packaging. Ultraviolet (UV) light is one example; it causes thymine dimers to form between adjacent thymines within a single strand of DNA ([\[link\]](#)). When DNA polymerase encounters the thymine dimer, it does not

always incorporate the appropriate complementary nucleotides (two adenines), and this leads to formation of mutations that can ultimately kill microorganisms.

UV light can be used effectively by both consumers and laboratory personnel to control microbial growth. UV lamps are now commonly incorporated into water purification systems for use in homes. In addition, small portable UV lights are commonly used by campers to purify water from natural environments before drinking. Germicidal lamps are also used in surgical suites, biological safety cabinets, and transfer hoods, typically emitting UV light at a wavelength of 260 nm. Because UV light does not penetrate surfaces and will not pass through plastics or glass, cells must be exposed directly to the light source.

Sunlight has a very broad spectrum that includes UV and visible light. In some cases, sunlight can be effective against certain bacteria because of both the formation of thymine dimers by UV light and by the production of reactive oxygen products induced in low amounts by exposure to visible light.



- (a) UV radiation causes the formation of thymine dimers in DNA, leading to lethal mutations in the exposed microbes. (b) Germicidal lamps that emit UV light are commonly used in the laboratory to sterilize equipment.

Note:

- What are two advantages of ionizing radiation as a sterilization method?
- How does the effectiveness of ionizing radiation compare with that of nonionizing radiation?

Note:**Irradiated Food: Would You Eat That?**

Of all the ways to prevent food spoilage and foodborne illness, gamma irradiation may be the most unappetizing. Although gamma irradiation is a proven method of eliminating potentially harmful microbes from food, the public has yet to buy in. Most of their concerns, however, stem from misinformation and a poor understanding of the basic principles of radiation. The most common method of irradiation is to expose food to cobalt-60 or cesium-137 by passing it through a radiation chamber on a conveyor belt. The food does not directly contact the radioactive material and does not become radioactive itself. Thus, there is no risk for exposure to radioactive material through eating gamma-irradiated foods. Additionally, irradiated foods are not significantly altered in terms of nutritional quality, aside from the loss of certain vitamins, which is also exacerbated by extended storage. Alterations in taste or smell may occur in irradiated foods with high fat content, such as fatty meats and dairy products, but this effect can be minimized by using lower doses of radiation at colder temperatures.

In the United States, the CDC, Environmental Protection Agency (EPA), and the Food and Drug Administration (FDA) have deemed irradiation safe and effective for various types of meats, poultry, shellfish, fresh fruits and vegetables, eggs with shells, and spices and seasonings. Gamma irradiation of foods has also been approved for use in many other countries, including France, the Netherlands, Portugal, Israel, Russia, China, Thailand, Belgium, Australia, and South Africa. To help ameliorate consumer concern and assist with education efforts, irradiated foods are now clearly labeled and marked with the international irradiation symbol, called the “radura” ([\[link\]](#)). Consumer acceptance seems to be rising, as indicated by several recent studies.[\[footnote\]](#)

AM Johnson et al. “Consumer Acceptance of Electron-Beam Irradiated Ready-to-Eat Poultry Meats.” *Food Processing Preservation*, 28 no. 4

(2004):302–319.



(a)



(b)

(a) Foods are exposed to gamma radiation by passage on a conveyor belt through a radiation chamber. (b) Gamma-irradiated foods must be clearly labeled and display the irradiation symbol, known as the “radura.”
(credit a, b: modification of work by U.S. Department of Agriculture)

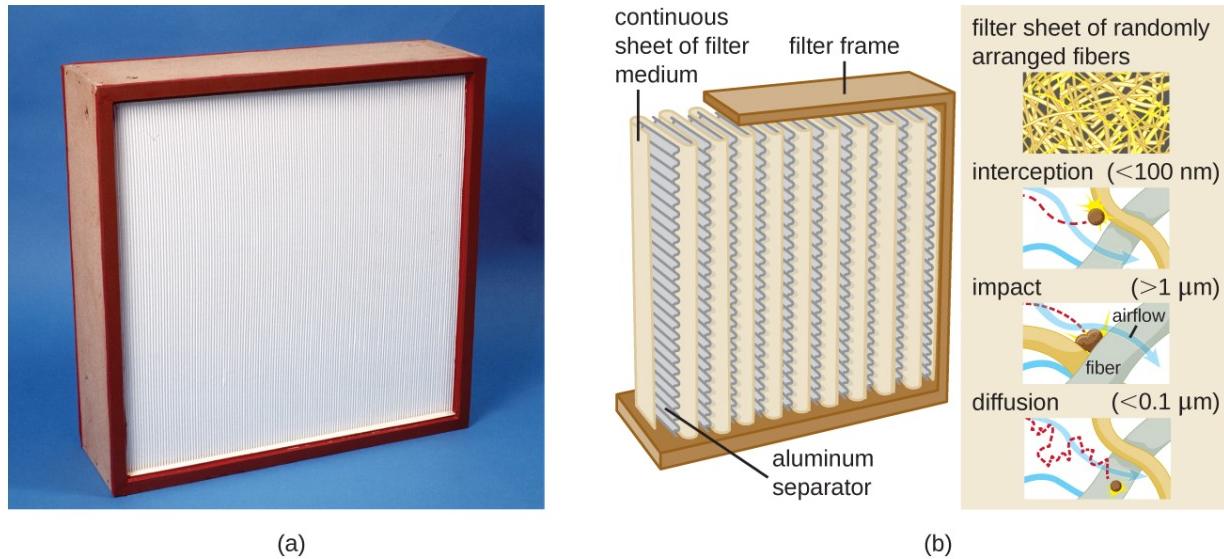
Sonication

The use of high-frequency ultrasound waves to disrupt cell structures is called **sonication**. Application of ultrasound waves causes rapid changes in pressure within the intracellular liquid; this leads to cavitation, the formation of bubbles inside the cell, which can disrupt cell structures and eventually cause the cell to lyse or collapse. Sonication is useful in the laboratory for efficiently lysing cells to release their contents for further research; outside the laboratory, sonication is used for cleaning surgical instruments, lenses, and a variety of other objects such as coins, tools, and musical instruments.

Filtration

Filtration is a method of physically separating microbes from samples. Air is commonly filtered through **high-efficiency particulate air (HEPA) filters**

([\[link\]](#)). HEPA filters have effective pore sizes of $0.3\text{ }\mu\text{m}$, small enough to capture bacterial cells, endospores, and many viruses, as air passes through these filters, nearly sterilizing the air on the other side of the filter. HEPA filters have a variety of applications and are used widely in clinical settings, in cars and airplanes, and even in the home. For example, they may be found in vacuum cleaners, heating and air-conditioning systems, and air purifiers.



(a) HEPA filters like this one remove microbes, endospores, and viruses as air flows through them. (b) A schematic of a HEPA filter. (credit a: modification of work by CSIRO; credit b: modification of work by "LadyofHats"/Mariana Ruiz Villareal)

Biological Safety Cabinets

Biological safety cabinets are a good example of the use of HEPA filters. HEPA filters in biological safety cabinets (BSCs) are used to remove particulates in the air either entering the cabinet (air intake), leaving the cabinet (air exhaust), or treating both the intake and exhaust. Use of an air-intake HEPA filter prevents environmental contaminants from entering the BSC, creating a clean area for handling biological materials. Use of an air-

exhaust HEPA filter prevents laboratory pathogens from contaminating the laboratory, thus maintaining a safe work area for laboratory personnel.

There are three classes of BSCs: I, II, and III. Each class is designed to provide a different level of protection for laboratory personnel and the environment; BSC II and III are also designed to protect the materials or devices in the cabinet. [\[link\]](#) summarizes the level of safety provided by each class of BSC for each BSL.

Biological Risks and BSCs				
Biological Risk Assessed	BSC Class	Protection of Personnel	Protection of Environment	Protection of Product
BSL-1, BSL-2, BSL-3	I	Yes	Yes	No
BSL-1, BSL-2, BSL-3	II	Yes	Yes	Yes
BSL-4	III; II when used in suit room with suit	Yes	Yes	Yes

Class I BSCs protect laboratory workers and the environment from a low to moderate risk for exposure to biological agents used in the laboratory. Air is drawn into the cabinet and then filtered before exiting through the building's exhaust system. Class II BSCs use directional air flow and partial barrier systems to contain infectious agents. Class III BSCs are designed for working with highly infectious agents like those used in BSL-4 laboratories. They are gas tight, and materials entering or exiting the cabinet must be passed through a double-door system, allowing the intervening space to be decontaminated between uses. All air is passed through one or two HEPA filters and an air incineration system before being exhausted directly to the outdoors (not through the building's exhaust system). Personnel can manipulate materials inside the Class III cabinet by using long rubber gloves sealed to the cabinet.

Note:



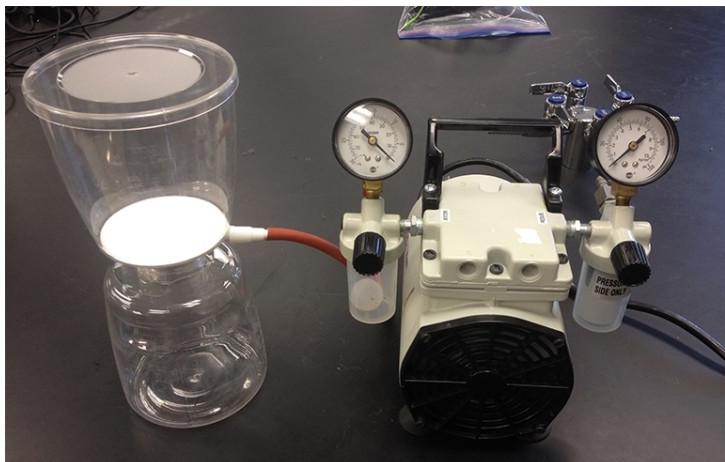
This [video](#) shows how BSCs are designed and explains how they protect personnel, the environment, and the product.

Filtration in Hospitals

HEPA filters are also commonly used in hospitals and surgical suites to prevent contamination and the spread of airborne microbes through ventilation systems. HEPA filtration systems may be designed for entire buildings or for individual rooms. For example, burn units, operating rooms, or isolation units may require special HEPA-filtration systems to remove opportunistic pathogens from the environment because patients in these rooms are particularly vulnerable to infection.

Membrane Filters

Filtration can also be used to remove microbes from liquid samples using **membrane filtration**. Membrane filters for liquids function similarly to HEPA filters for air. Typically, membrane filters that are used to remove bacteria have an effective pore size of 0.2 μm , smaller than the average size of a bacterium (1 μm), but filters with smaller pore sizes are available for more specific needs. Membrane filtration is useful for removing bacteria from various types of heat-sensitive solutions used in the laboratory, such as antibiotic solutions and vitamin solutions. Large volumes of culture media may also be filter sterilized rather than autoclaved to protect heat-sensitive components. Often when filtering small volumes, syringe filters are used, but vacuum filters are typically used for filtering larger volumes ([\[link\]](#)).



(a)



(b)

Membrane filters come in a variety of sizes, depending on the volume of solution being filtered. (a) Larger volumes are filtered in units like these.

The solution is drawn through the filter by connecting the unit to a vacuum. (b) Smaller volumes are often filtered using syringe filters, which are units that fit on the end of a syringe. In this case, the solution is pushed through by depressing the syringe's plunger. (credit a, b: modification of work by Brian Forster)

Note:

- Would membrane filtration with a 0.2- μm filter likely remove viruses from a solution? Explain.
- Name at least two common uses of HEPA filtration in clinical or laboratory settings.

[link] and [link] summarize the physical methods of control discussed in this section.

Physical Methods of Control			
Method	Conditions	Mode of Action	Example Uses
Heat			
Boiling	100 °C at sea level	Denatures proteins and alters membranes	Cooking, personal use, preparing certain laboratory media
Dry-heat oven	170 °C for 2 hours	Denatures proteins and alters membranes, dehydration, desiccation	Sterilization of heat-stable medical and laboratory equipment and glassware
Incineration	Exposure to flame	Destroy by burning	Flaming loop, microincinerator
Autoclave	Typical settings: 121 °C for 15 minutes at 15 pounds per square inch (psi)	Denatures proteins and alters membranes	Sterilization of microbiological media, heat-stable medical and laboratory equipment, and other heat-stable items
Pasteurization	Can vary. One type is 72 °C for 15 seconds (HTST)	Denatures proteins and alters membranes	Prevents spoilage of milk, apple juice, honey, and other ingestible liquids
Cold			
Refrigeration	0 °C to 7 °C	Inhibits metabolism (slows or arrests cell division)	Preservation of food or laboratory materials (solutions, cultures)
Freezing	Below –2 °C	Stops metabolism, may kill microbes	Long-term storage of food, laboratory cultures, or medical specimens
Pressure			
High-pressure processing	100–800 MPa	Denatures proteins and can cause cell lysis	Preservation of food
Hyperbaric oxygen therapy	Air pressure three times higher than normal	Inhibits metabolism and growth of anaerobic microbes	Treatment of certain infections (e.g., gas gangrene)
Desiccation			
Simple desiccation	Drying	Inhibits metabolism	Dried fruits, jerky
Reduce water activity	Addition of salt or water	Inhibits metabolism and can cause lysis	Salted meats and fish, honey, jams and jellies
Lyophilization	Rapid freezing under vacuum	Inhibits metabolism	Preservation of food, laboratory cultures, or reagents
Radiation			
Ionizing radiation	Exposure to X-rays or gamma rays	Alters molecular structures, introduces double-strand breaks into DNA	Sterilization of spices and heat-sensitive laboratory and medical items; used for food sterilization in Europe but not widely accepted in US
Nonionizing radiation	Exposure to ultraviolet light	Introduces thymine dimers, leading to mutations	Surface sterilization of laboratory materials, water purification

Physical Methods of Control (continued)			
Method	Conditions	Mode of Action	Example Uses
Sonication			
Sonication	Exposure to ultrasonic waves	Cavitation (formation of empty space) disrupts cells, lysing them	Laboratory research to lyse cells; cleaning jewelry, lenses, and equipment
Filtration			
HEPA filtration	Use of high-efficiency particulate air (HEPA) filter with 0.3 μm pore size	Physically removes microbes from air	Laboratory biological safety cabinets, operating rooms, isolation units, heating and air conditioning systems, vacuum cleaners
Membrane filtration	Use of membrane filter with 0.2- μm or smaller pore size	Physically removes microbes from liquid solutions	Removal of bacteria from heat-sensitive solutions like vitamins, antibiotics, and media with heat-sensitive components

Key Concepts and Summary

- Heat is a widely used and highly effective method for controlling microbial growth.
- **Dry-heat sterilization** protocols are used commonly in aseptic techniques in the laboratory. However, **moist-heat sterilization** is typically the more effective protocol because it penetrates cells better than dry heat does.
- **Pasteurization** is used to kill pathogens and reduce the number of microbes that cause food spoilage. **High-temperature, short-time pasteurization** is commonly used to pasteurize milk that will be refrigerated; **ultra-high temperature pasteurization** can be used to pasteurize milk for long-term storage without refrigeration.
- Refrigeration slows microbial growth; freezing stops growth, killing some organisms. Laboratory and medical specimens may be frozen on dry ice or at ultra-low temperatures for storage and transport.
- High-pressure processing can be used to kill microbes in food. Hyperbaric oxygen therapy to increase oxygen saturation has also been used to treat certain infections.
- **Desiccation** has long been used to preserve foods and is accelerated through the addition of salt or sugar, which decrease water activity in foods.
- **Lyophilization** combines cold exposure and desiccation for the long-term storage of foods and laboratory materials, but microbes remain and can be

rehydrated.

- **Ionizing radiation**, including gamma irradiation, is an effective way to sterilize heat-sensitive and packaged materials. **Nonionizing radiation**, like ultraviolet light, is unable to penetrate surfaces but is useful for surface sterilization.
- **HEPA** filtration is commonly used in hospital ventilation systems and biological safety cabinets in laboratories to prevent transmission of airborne microbes. **Membrane filtration** is commonly used to remove bacteria from heat-sensitive solutions.

Short Answer

Exercise:

Problem:

What is the advantage of HTST pasteurization compared with sterilization? What is an advantage of UHT treatment?

Exercise:

Problem: How does the addition of salt or sugar help preserve food?

Exercise:

Problem:

Which is more effective at killing microbes: autoclaving or freezing?
Explain.

Critical Thinking

Exercise:

Problem:

In 2001, endospores of *Bacillus anthracis*, the causative agent of anthrax, were sent to government officials and news agencies via the mail. In response, the US Postal Service began to irradiate mail with UV light. Was this an effective strategy? Why or why not?

Using Chemicals to Control Microorganisms

LEARNING OBJECTIVES

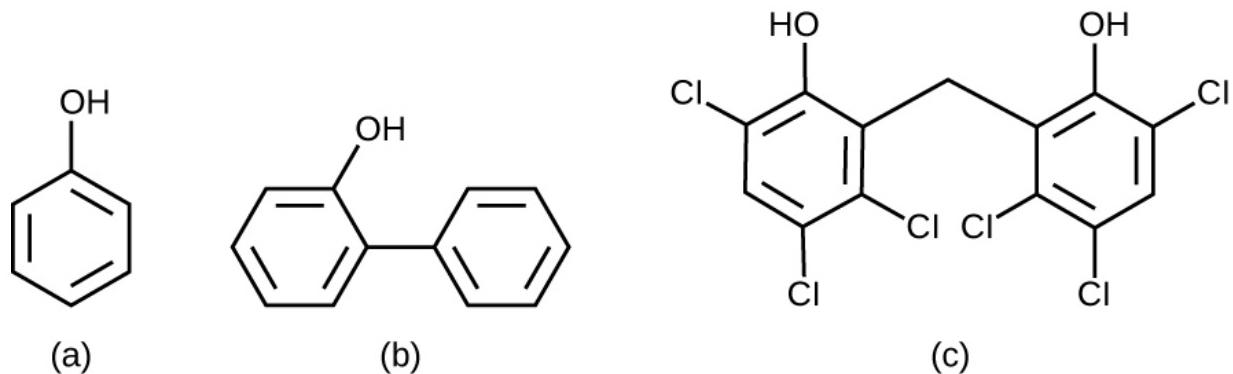
- Understand and compare various chemicals used to control microbial growth, including their uses, advantages and disadvantages, and mode of action

In addition to physical methods of microbial control, chemicals are also used to control microbial growth. A wide variety of chemicals can be used as disinfectants or antiseptics. When choosing which to use, it is important to consider the type of microbe targeted; how clean the item needs to be; the disinfectant's effect on the item's integrity; its safety to animals, humans, and the environment; its expense; and its ease of use. This section describes the variety of chemicals used as disinfectants and antiseptics, including their mechanisms of action and common uses.

Phenolics

In the 1800s, scientists began experimenting with a variety of chemicals for disinfection. In the 1860s, British surgeon Joseph Lister (1827–1912) began using carbolic acid, known as phenol, as a disinfectant for the treatment of surgical wounds (see [Foundations of Modern Cell Theory](#)). In 1879, Lister's work inspired the American chemist Joseph Lawrence (1836–1909) to develop Listerine, an alcohol-based mixture of several related compounds that is still used today as an oral antiseptic. Today, carbolic acid is no longer used as a surgical disinfectant because it is a skin irritant, but the chemical compounds found in antiseptic mouthwashes and throat lozenges are called **phenolics**.

Chemically, phenol consists of a benzene ring with an –OH group, and phenolics are compounds that have this group as part of their chemical structure ([\[link\]](#)). Phenolics such as thymol and eucalyptol occur naturally in plants. Other phenolics can be derived from creosote, a component of coal tar. Phenolics tend to be stable, persistent on surfaces, and less toxic than phenol. They inhibit microbial growth by denaturing proteins and disrupting membranes.



Phenol and phenolic compounds have been used to control microbial growth. (a) Chemical structure of phenol, also known as carbolic acid. (b) o-Phenylphenol, a type of phenolic, has been used as a disinfectant as well as to control bacterial and fungal growth on harvested citrus fruits. (c) Hexachlorophene, another phenol, known as a bisphenol (two rings), is the active ingredient in pHisoHex.

Since Lister's time, several phenolic compounds have been used to control microbial growth. Phenolics like cresols (methylated phenols) and o-phenylphenol were active ingredients in various formulations of Lysol since its invention in 1889. o-Phenylphenol was also commonly used in agriculture to control bacterial and fungal growth on harvested crops, especially citrus fruits, but its use in the United States is now far more limited. The bisphenol hexachlorophene, a disinfectant, is the active ingredient in pHisoHex, a topical cleansing detergent widely used for handwashing in hospital settings. pHisoHex is particularly effective against

gram-positive bacteria, including those causing staphylococcal and streptococcal skin infections. pHisoHex was formerly used for bathing infants, but this practice has been discontinued because it has been shown that exposure to hexachlorophene can lead to neurological problems.

Triclosan is another bisphenol compound that has seen widespread application in antibacterial products over the last several decades. Initially used in toothpastes, triclosan is now commonly used in hand soaps and is frequently impregnated into a wide variety of other products, including cutting boards, knives, shower curtains, clothing, and concrete, to make them antimicrobial. It is particularly effective against gram-positive bacteria on the skin, as well as certain gram-negative bacteria and yeasts.[\[footnote\]](#) US Food and Drug Administration. “Triclosan: What Consumers Should Know.” 2015.

<http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm205999.htm>. Accessed June 9, 2016.

Note:

Triclosan: Antibacterial Overkill?

Hand soaps and other cleaning products are often marketed as “antibacterial,” suggesting that they provide a level of cleanliness superior to that of conventional soaps and cleansers. But are the antibacterial ingredients in these products really safe and effective?

About 75% of antibacterial liquid hand soaps and 30% of bar soaps contain the chemical triclosan, a phenolic, ([\[link\]](#)).[\[footnote\]](#) Triclosan blocks an enzyme in the bacterial fatty acid-biosynthesis pathway that is not found in the comparable human pathway. Although the use of triclosan in the home increased dramatically during the 1990s, more than 40 years of research by the FDA have turned up no conclusive evidence that washing with triclosan-containing products provides increased health benefits compared with washing with traditional soap. Although some studies indicate that fewer bacteria may remain on a person’s hands after washing with triclosan-based soap, compared with traditional soap, no evidence points to any reduction in the transmission of bacteria that cause respiratory and

gastrointestinal illness. In short, soaps with triclosan may remove or kill a few more germs but not enough to reduce the spread of disease.

J. Stromberg. "Five Reasons Why You Should Probably Stop Using Antibacterial Soap." *Smithsonian.com* January 3, 2014.

<http://www.smithsonianmag.com/science-nature/five-reasons-why-you-should-probably-stop-using-antibacterial-soap-180948078/?no-ist>.

Accessed June 9, 2016.

Perhaps more disturbing, some clear risks associated with triclosan-based soaps have come to light. The widespread use of triclosan has led to an increase in triclosan-resistant bacterial strains, including those of clinical importance, such as *Salmonella enterica*; this resistance may render triclosan useless as an antibacterial in the long run.[\[footnote\]](#)[\[footnote\]](#)

Bacteria can easily gain resistance to triclosan through a change to a single gene encoding the targeted enzyme in the bacterial fatty acid-synthesis pathway. Other disinfectants with a less specific mode of action are much less prone to engendering resistance because it would take much more than a single genetic change.

SP Yazdankhah et al. "Triclosan and Antimicrobial Resistance in Bacteria: An Overview." *Microbial Drug Resistance* 12 no. 2 (2006):83–90.

L. Birošová, M. Mikulášová. "Development of Triclosan and Antibiotic Resistance in *Salmonella enterica* serovar Typhimurium." *Journal of Medical Microbiology* 58 no. 4 (2009):436–441.

Use of triclosan over the last several decades has also led to a buildup of the chemical in the environment. Triclosan in hand soap is directly introduced into wastewater and sewage systems as a result of the handwashing process. There, its antibacterial properties can inhibit or kill bacteria responsible for the decomposition of sewage, causing septic systems to clog and back up. Eventually, triclosan in wastewater finds its way into surface waters, streams, lakes, sediments, and soils, disrupting natural populations of bacteria that carry out important environmental functions, such as inhibiting algae. Triclosan also finds its way into the bodies of amphibians and fish, where it can act as an endocrine disruptor. Detectable levels of triclosan have also been found in various human bodily fluids, including breast milk, plasma, and urine.[\[footnote\]](#) In fact, a study conducted by the CDC found detectable levels of triclosan in the urine of 75% of 2,517 people tested in 2003–2004.[\[footnote\]](#) This finding

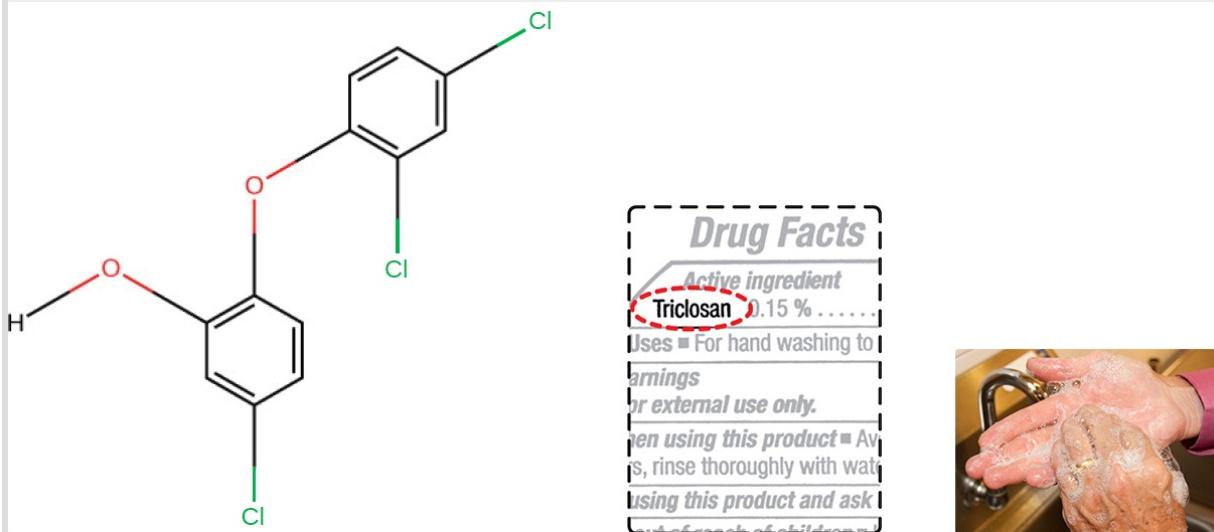
is even more troubling given the evidence that triclosan may affect immune function in humans.[\[footnote\]](#)

AB Dann, A. Hontela. "Triclosan: Environmental Exposure, Toxicity and Mechanisms of Action." *Journal of Applied Toxicology* 31 no. 4 (2011):285–311.

US Centers for Disease Control and Prevention. "Triclosan Fact Sheet." 2013. http://www.cdc.gov/biomonitoring/Triclosan_FactSheet.html. Accessed June 9, 2016.

EM Clayton et al. "The Impact of Bisphenol A and Triclosan on Immune Parameters in the US Population, NHANES 2003-2006." *Environmental Health Perspectives* 119 no. 3 (2011):390.

In December 2013, the FDA gave soap manufacturers until 2016 to prove that antibacterial soaps provide a significant benefit over traditional soaps; if unable to do so, manufacturers will be forced to remove these products from the market.



Triclosan is a common ingredient in antibacterial soaps despite evidence that it poses environmental and health risks and offers no significant health benefit compared to conventional soaps. (credit b, c: modification of work by FDA)

Note:

- Why is triclosan more like an antibiotic than a traditional disinfectant?

Heavy Metals

Some of the first chemical disinfectants and antiseptics to be used were heavy metals. Heavy metals kill microbes by binding to proteins, thus inhibiting enzymatic activity ([\[link\]](#)). Heavy metals are oligodynamic, meaning that very small concentrations show significant antimicrobial activity. Ions of heavy metals bind to sulfur-containing amino acids strongly and bioaccumulate within cells, allowing these metals to reach high localized concentrations. This causes proteins to denature.

Heavy metals are not selectively toxic to microbial cells. They may bioaccumulate in human or animal cells, as well, and excessive concentrations can have toxic effects on humans. If too much silver accumulates in the body, for example, it can result in a condition called argyria, in which the skin turns irreversibly blue-gray. One way to reduce the potential toxicity of heavy metals is by carefully controlling the duration of exposure and concentration of the heavy metal.



(a)



(b)



(c)



(d)



(e)

Heavy metals denature proteins, impairing cell function and, thus, giving them strong antimicrobial properties. (a) Copper in fixtures like

this door handle kills microbes that otherwise might accumulate on frequently touched surfaces. (b) Eating utensils contain small amounts of silver to inhibit microbial growth. (c) Copper commonly lines incubators to minimize contamination of cell cultures stored inside. (d) Antiseptic mouthwashes commonly contain zinc chloride. (e) This patient is suffering from argyria, an irreversible condition caused by bioaccumulation of silver in the body. (credit b: modification of work by “Shoshanah”/Flickr; credit e: modification of work by Herbert L. Fred and Hendrik A. van Dijk)

Mercury

Mercury is an example of a heavy metal that has been used for many years to control microbial growth. It was used for many centuries to treat syphilis. Mercury compounds like mercuric chloride are mainly bacteriostatic and have a very broad spectrum of activity. Various forms of mercury bind to sulfur-containing amino acids within proteins, inhibiting their functions.

In recent decades, the use of such compounds has diminished because of mercury’s toxicity. It is toxic to the central nervous, digestive, and renal systems at high concentrations, and has negative environmental effects, including bioaccumulation in fish. Topical antiseptics such as mercurochrome, which contains mercury in low concentrations, and merthiolate, a **tincture** (a solution of mercury dissolved in alcohol) were once commonly used. However, because of concerns about using mercury compounds, these antiseptics are no longer sold in the United States.

Silver

Silver has long been used as an antiseptic. In ancient times, drinking water was stored in silver jugs.[\[footnote\]](#) Silvadene cream is commonly used to treat topical wounds and is particularly helpful in preventing infection in

burn wounds. Silver nitrate drops were once routinely applied to the eyes of newborns to protect against ophthalmia neonatorum, eye infections that can occur due to exposure to pathogens in the birth canal, but antibiotic creams are more now commonly used. Silver is often combined with antibiotics, making the antibiotics thousands of times more effective.[\[footnote\]](#) Silver is also commonly incorporated into catheters and bandages, rendering them antimicrobial; however, there is evidence that heavy metals may also enhance selection for antibiotic resistance.[\[footnote\]](#)

N. Silvestry-Rodriguez et al. "Silver as a Disinfectant." In *Reviews of Environmental Contamination and Toxicology*, pp. 23-45. Edited by GW Ware and DM Whitacre. New York: Springer, 2007.

B. Owens. "Silver Makes Antibiotics Thousands of Times More Effective." *Nature* June 19 2013. <http://www.nature.com/news/silver-makes-antibiotics-thousands-of-times-more-effective-1.13232>

C. Seiler, TU Berendonk. "Heavy Metal Driven Co-Selection of Antibiotic Resistance in Soil and Water Bodies Impacted by Agriculture and Aquaculture." *Frontiers in Microbiology* 3 (2012):399.

Copper, Nickel, and Zinc

Several other heavy metals also exhibit antimicrobial activity. Copper sulfate is a common algicide used to control algal growth in swimming pools and fish tanks. The use of metallic copper to minimize microbial growth is also becoming more widespread. Copper linings in incubators help reduce contamination of cell cultures. The use of copper pots for water storage in underdeveloped countries is being investigated as a way to combat diarrheal diseases. Copper coatings are also becoming popular for frequently handled objects such as doorknobs, cabinet hardware, and other fixtures in health-care facilities in an attempt to reduce the spread of microbes.

Nickel and zinc coatings are now being used in a similar way. Other forms of zinc, including zinc chloride and zinc oxide, are also used commercially. Zinc chloride is quite safe for humans and is commonly found in mouthwashes, substantially increasing their length of effectiveness. Zinc oxide is found in a variety of products, including topical antiseptic creams

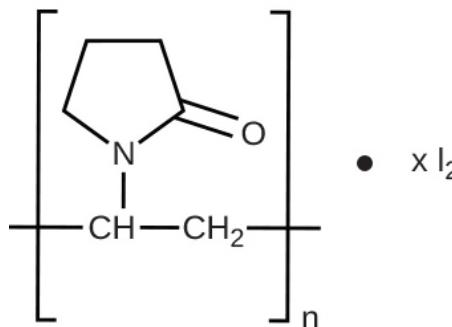
such as calamine lotion, diaper ointments, baby powder, and dandruff shampoos.

Note:

- Why are many heavy metals both antimicrobial and toxic to humans?

Halogens

Other chemicals commonly used for disinfection are the halogens iodine, chlorine, and fluorine. Iodine works by oxidizing cellular components, including sulfur-containing amino acids, nucleotides, and fatty acids, and destabilizing the macromolecules that contain these molecules. It is often used as a topical tincture, but it may cause staining or skin irritation. An **iodophor** is a compound of iodine complexed with an organic molecule, thereby increasing iodine's stability and, in turn, its efficacy. One common iodophor is povidone-iodine, which includes a wetting agent that releases iodine relatively slowly. Betadine is a brand of povidone-iodine commonly used as a hand scrub by medical personnel before surgery and for topical antisepsis of a patient's skin before incision ([\[link\]](#)).



(a)



(b)

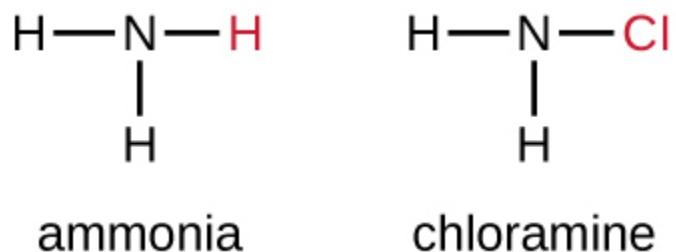
(a) Betadine is a solution of the iodophor povidone-iodine. (b) It is commonly used as a topical antiseptic on a patient's skin before incision during surgery. (credit b: modification of work by Andrew Ratto)

Chlorine is another halogen commonly used for disinfection. When chlorine gas is mixed with water, it produces a strong oxidant called hypochlorous acid, which is uncharged and enters cells easily. Chlorine gas is commonly used in municipal drinking water and wastewater treatment plants, with the resulting hypochlorous acid producing the actual antimicrobial effect. Those working at water treatment facilities need to take great care to minimize personal exposure to chlorine gas. Sodium hypochlorite is the chemical component of common household bleach, and it is also used for a wide variety of disinfecting purposes. Hypochlorite salts, including sodium and calcium hypochlorites, are used to disinfect swimming pools. Chlorine gas, sodium hypochlorite, and calcium hypochlorite are also commonly used disinfectants in the food processing and restaurant industries to reduce the spread of foodborne diseases. Workers in these industries also need to take care to use these products correctly to ensure their own safety as well as the safety of consumers. A recent joint statement published by the Food and Agriculture Organization (FAO) of the United Nations and WHO indicated that none of the many beneficial uses of chlorine products in food

processing to reduce the spread of foodborne illness posed risks to consumers.[\[footnote\]](#)

World Health Organization. “Benefits and Risks of the Use of Chlorine-Containing Disinfectants in Food Production and Food Processing: Report of a Joint FAO/WHO Expert Meeting.” Geneva, Switzerland: World Health Organization, 2009.

Another class of chlorinated compounds called chloramines are widely used as disinfectants. Chloramines are relatively stable, releasing chlorine over long periods time. Chloramines are derivatives of ammonia by substitution of one, two, or all three hydrogen atoms with chlorine atoms ([\[link\]](#)).



Monochloroamine, one of the chloramines, is derived from ammonia by the replacement of one hydrogen atom with a chlorine atom.

Chloramines and other chlorine compounds may be used for disinfection of drinking water, and chloramine tablets are frequently used by the military for this purpose. After a natural disaster or other event that compromises the public water supply, the CDC recommends disinfecting tap water by adding small amounts of regular household bleach. Recent research suggests that sodium dichloroisocyanurate (NaDCC) may also be a good alternative for drinking water disinfection. Currently, NaDCC tablets are available for general use and for use by the military, campers, or those with emergency needs; for these uses, NaDCC is preferable to chloramine tablets. Chlorine

dioxide, a gaseous agent used for fumigation and sterilization of enclosed areas, is also commonly used for the disinfection of water.

Although chlorinated compounds are relatively effective disinfectants, they have their disadvantages. Some may irritate the skin, nose, or eyes of some individuals, and they may not completely eliminate certain hardy organisms from contaminated drinking water. The fungus *Cryptosporidium*, for example, has a protective outer shell that makes it resistant to chlorinated disinfectants. Thus, boiling of drinking water in emergency situations is recommended when possible.

The halogen fluorine is also known to have antimicrobial properties that contribute to the prevention of dental caries (cavities).[\[footnote\]](#) Fluoride is the main active ingredient of toothpaste and is also commonly added to tap water to help communities maintain oral health. Chemically, fluoride can become incorporated into the hydroxyapatite of tooth enamel, making it more resistant to corrosive acids produced by the fermentation of oral microbes. Fluoride also enhances the uptake of calcium and phosphate ions in tooth enamel, promoting remineralization. In addition to strengthening enamel, fluoride also seems to be bacteriostatic. It accumulates in plaque-forming bacteria, interfering with their metabolism and reducing their production of the acids that contribute to tooth decay.

RE Marquis. “Antimicrobial Actions of Fluoride for Oral Bacteria.”

Canadian Journal of Microbiology 41 no. 11 (1995):955–964.

Note:

- What is a benefit of a chloramine over hypochlorite for disinfecting?

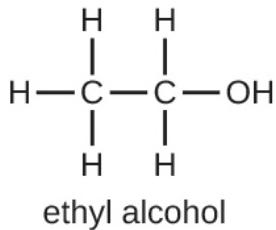
Alcohols

Alcohols make up another group of chemicals commonly used as disinfectants and antiseptics. They work by rapidly denaturing proteins,

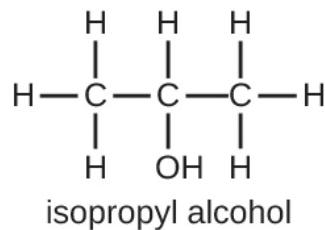
which inhibits cell metabolism, and by disrupting membranes, which leads to cell lysis. Once denatured, the proteins may potentially refold if enough water is present in the solution. Alcohols are typically used at concentrations of about 70% aqueous solution and, in fact, work better in aqueous solutions than 100% alcohol solutions. This is because alcohols coagulate proteins. In higher alcohol concentrations, rapid coagulation of surface proteins prevents effective penetration of cells. The most commonly used alcohols for disinfection are ethyl alcohol (ethanol) and isopropyl alcohol (isopropanol, rubbing alcohol) ([\[link\]](#)).

Alcohols tend to be bactericidal and fungicidal, but may also be viricidal for enveloped viruses only. Although alcohols are not sporicidal, they do inhibit the processes of sporulation and germination. Alcohols are volatile and dry quickly, but they may also cause skin irritation because they dehydrate the skin at the site of application. One common clinical use of alcohols is swabbing the skin for degerming before needle injection. Alcohols also are the active ingredients in instant hand sanitizers, which have gained popularity in recent years. The alcohol in these hand sanitizers works both by denaturing proteins and by disrupting the microbial cell membrane, but will not work effectively in the presence of visible dirt.

Last, alcohols are used to make tinctures with other antiseptics, such as the iodine tinctures discussed previously in this chapter. All in all, alcohols are inexpensive and quite effective for the disinfection of a broad range of vegetative microbes. However, one disadvantage of alcohols is their high volatility, limiting their effectiveness to immediately after application.



(a)



(b)

(a) Ethyl alcohol, the intoxicating ingredient found in alcoholic drinks, is also used commonly as a disinfectant. (b) Isopropyl alcohol, also called rubbing alcohol, has a related molecular structure and is another commonly used disinfectant. (credit a photo: modification of work by D Coetzee; credit b photo: modification of work by Craig Spurrier)

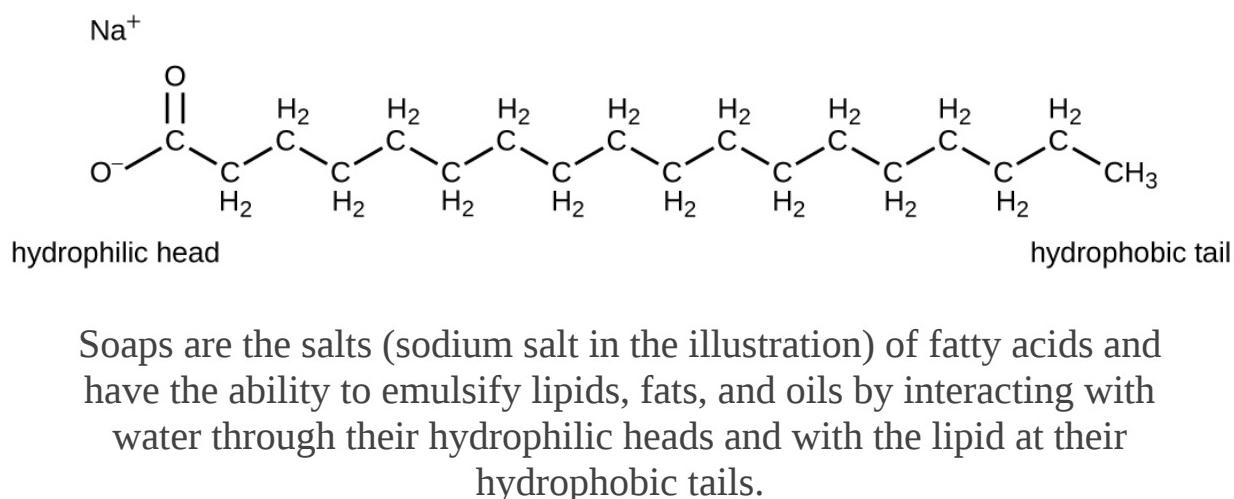
Note:

- Name at least three advantages of alcohols as disinfectants.
- Describe several specific applications of alcohols used in disinfectant products.

Surfactants

Surface-active agents, or **surfactants**, are a group of chemical compounds that lower the surface tension of water. Surfactants are the major ingredients in soaps and detergents. Soaps are salts of long-chain fatty acids and have both polar and nonpolar regions, allowing them to interact with polar and

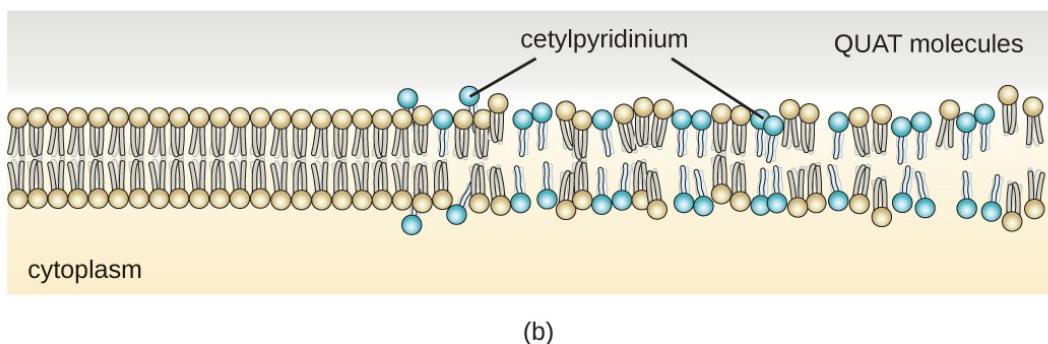
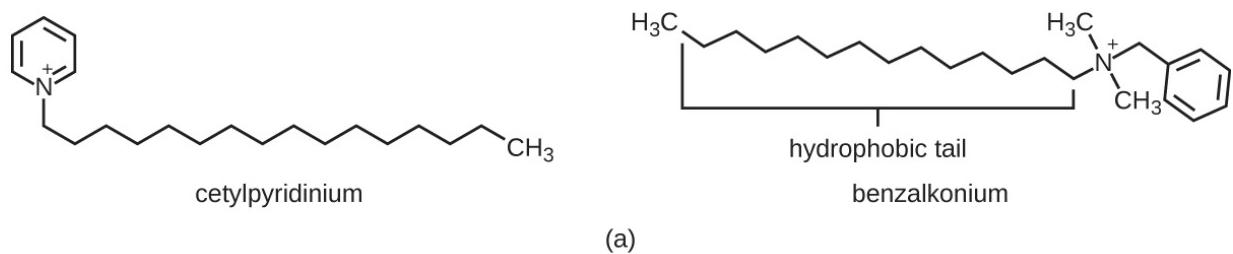
nonpolar regions in other molecules ([\[link\]](#)). They can interact with nonpolar oils and grease to create emulsions in water, loosening and lifting away dirt and microbes from surfaces and skin. Soaps do not kill or inhibit microbial growth and so are not considered antiseptics or disinfectants. However, proper use of soaps mechanically carries away microorganisms, effectively degirming a surface. Some soaps contain added bacteriostatic agents such as triclocarban or cloflucarban, compounds structurally related to triclosan, that introduce antiseptic or disinfectant properties to the soaps.



Soaps are the salts (sodium salt in the illustration) of fatty acids and have the ability to emulsify lipids, fats, and oils by interacting with water through their hydrophilic heads and with the lipid at their hydrophobic tails.

Soaps, however, often form films that are difficult to rinse away, especially in hard water, which contains high concentrations of calcium and magnesium mineral salts. Detergents contain synthetic surfactant molecules with both polar and nonpolar regions that have strong cleansing activity but are more soluble, even in hard water, and, therefore, leave behind no soapy deposits. Anionic detergents, such as those used for laundry, have a negatively charged anion at one end attached to a long hydrophobic chain, whereas cationic detergents have a positively charged cation instead. Cationic detergents include an important class of disinfectants and antiseptics called the **quaternary ammonium salts (quats)**, named for the characteristic quaternary nitrogen atom that confers the positive charge ([\[link\]](#)). Overall, quats have properties similar to phospholipids, having hydrophilic and hydrophobic ends. As such, quats have the ability to insert

into the bacterial phospholipid bilayer and disrupt membrane integrity. The cationic charge of quats appears to confer their antimicrobial properties, which are diminished when neutralized. Quats have several useful properties. They are stable, nontoxic, inexpensive, colorless, odorless, and tasteless. They tend to be bactericidal by disrupting membranes. They are also active against fungi, protozoans, and enveloped viruses, but endospores are unaffected. In clinical settings, they may be used as antiseptics or to disinfect surfaces. Mixtures of quats are also commonly found in household cleaners and disinfectants, including many current formulations of Lysol brand products, which contain benzalkonium chlorides as the active ingredients. Benzalkonium chlorides, along with the quat cetylpyrimidine chloride, are also found in products such as skin antiseptics, oral rinses, and mouthwashes.



- (a) Two common quats are benzylalkonium chloride and cetylpyrimidine chloride. Note the hydrophobic nonpolar carbon chain at one end and the nitrogen-containing cationic component at the other end. (b) Quats are able to infiltrate the phospholipid plasma membranes of bacterial cells and disrupt their integrity, leading to death of the cell.

Note:

- Why are soaps not considered disinfectants?

Note:

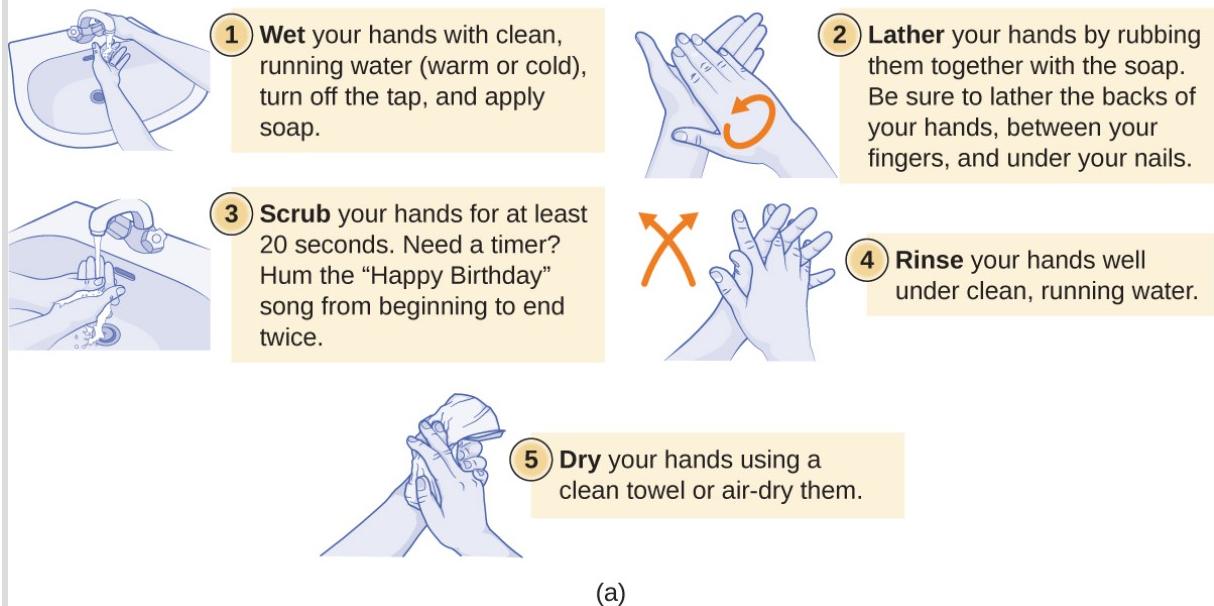
Handwashing the Right Way

Handwashing is critical for public health and should be emphasized in a clinical setting. For the general public, the CDC recommends handwashing before, during, and after food handling; before eating; before and after interacting with someone who is ill; before and after treating a wound; after using the toilet or changing diapers; after coughing, sneezing, or blowing the nose; after handling garbage; and after interacting with an animal, its feed, or its waste. [\[link\]](#) illustrates the five steps of proper handwashing recommended by the CDC.

Handwashing is even more important for health-care workers, who should wash their hands thoroughly between every patient contact, after the removal of gloves, after contact with bodily fluids and potentially infectious fomites, and before and after assisting a surgeon with invasive procedures. Even with the use of proper surgical attire, including gloves, scrubbing for surgery is more involved than routine handwashing. The goal of surgical scrubbing is to reduce the normal microbiota on the skin's surface to prevent the introduction of these microbes into a patient's surgical wounds.

There is no single widely accepted protocol for surgical scrubbing. Protocols for length of time spent scrubbing may depend on the antimicrobial used; health-care workers should always check the manufacturer's recommendations. According to the Association of Surgical Technologists (AST), surgical scrubs may be performed with or without the use of brushes ([\[link\]](#)).

CDC handwashing recommendations for the general public



(a)



(b)

(a) The CDC recommends five steps as part of typical handwashing for the general public. (b) Surgical scrubbing is more extensive, requiring scrubbing starting from the fingertips, extending to the hands and forearms, and then up beyond the elbows, as shown here.
(credit a: modification of work by World Health Organization)

Note:

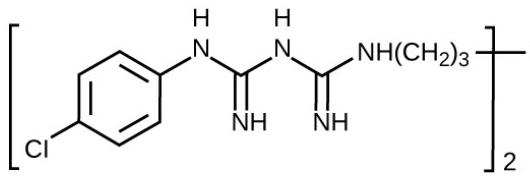


To [learn more](#) about proper handwashing, visit the CDC's website.

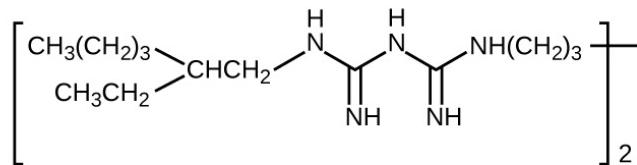
Bisbiguanides

Bisbiguanides were first synthesized in the 20th century and are cationic (positively charged) molecules known for their antiseptic properties ([\[link\]](#)). One important **bisbiguanide** antiseptic is chlorhexidine. It has broad-spectrum activity against yeasts, gram-positive bacteria, and gram-negative bacteria, with the exception of *Pseudomonas aeruginosa*, which may develop resistance on repeated exposure. [\[footnote\]](#) Chlorhexidine disrupts cell membranes and is bacteriostatic at lower concentrations or bactericidal at higher concentrations, in which it actually causes the cells' cytoplasmic contents to congeal. It also has activity against enveloped viruses. However, chlorhexidine is poorly effective against *Mycobacterium tuberculosis* and nonenveloped viruses, and it is not sporicidal. Chlorhexidine is typically used in the clinical setting as a surgical scrub and for other handwashing needs for medical personnel, as well as for topical antisepsis for patients before surgery or needle injection. It is more persistent than iodophors, providing long-lasting antimicrobial activity. Chlorhexidine solutions may also be used as oral rinses after oral procedures or to treat gingivitis. Another bisbiguanide, alexidine, is gaining popularity as a surgical scrub and an oral rinse because it acts faster than chlorhexidine.

L. Thomas et al. “Development of Resistance to Chlorhexidine Diacetate in *Pseudomonas aeruginosa* and the Effect of a ‘Residual’ Concentration.” *Journal of Hospital Infection* 46 no. 4 (2000):297–303.



chlorhexidine



alexidine

The bisbiguanides chlorhexidine and alexidine are cationic antiseptic compounds commonly used as surgical scrubs.

Note:

- What two effects does chlorhexidine have on bacterial cells?

Alkylating Agents

The **alkylating agents** are a group of strong disinfecting chemicals that act by replacing a hydrogen atom within a molecule with an alkyl group ($\text{C}_n\text{H}_{2n+1}$), thereby inactivating enzymes and nucleic acids ([\[link\]](#)). The alkylating agent formaldehyde (CH_2OH) is commonly used in solution at a concentration of 37% (known as formalin) or as a gaseous disinfectant and biocide. It is a strong, broad-spectrum disinfectant and biocide that has the ability to kill bacteria, viruses, fungi, and endospores, leading to sterilization at low temperatures, which is sometimes a convenient alternative to the more labor-intensive heat sterilization methods. It also cross-links proteins and has been widely used as a chemical fixative. Because of this, it is used for the storage of tissue specimens and as an embalming fluid. It also has been used to inactivate infectious agents in vaccine preparation. Formaldehyde is very irritating to living tissues and is also carcinogenic; therefore, it is not used as an antiseptic.

Glutaraldehyde is structurally similar to formaldehyde but has two reactive aldehyde groups, allowing it to act more quickly than formaldehyde. It is commonly used as a 2% solution for sterilization and is marketed under the brand name Cidex. It is used to disinfect a variety of surfaces and surgical and medical equipment. However, similar to formaldehyde, glutaraldehyde irritates the skin and is not used as an antiseptic.

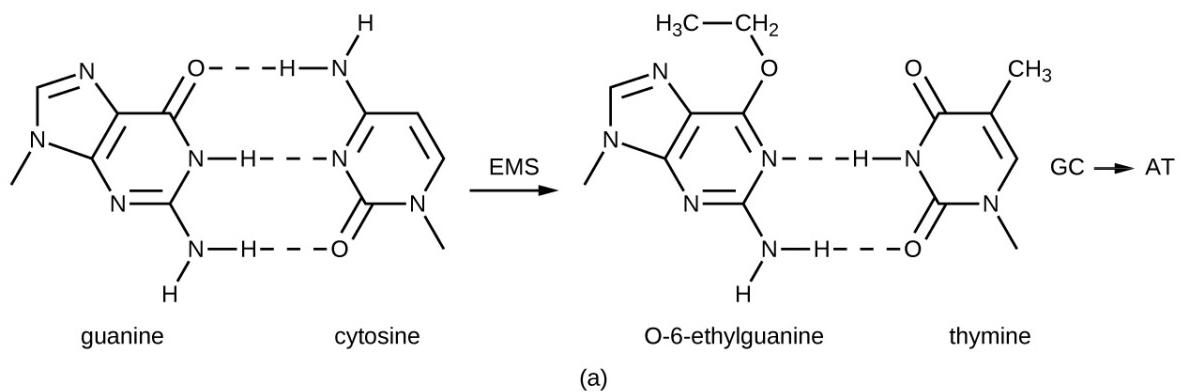
A new type of disinfectant gaining popularity for the disinfection of medical equipment is o-phthalaldehyde (OPA), which is found in some newer formulations of Cidex and similar products, replacing glutaraldehyde. o-Phthalaldehyde also has two reactive aldehyde groups, but they are linked by an aromatic bridge. o-Phthalaldehyde is thought to work similarly to glutaraldehyde and formaldehyde, but is much less irritating to skin and nasal passages, produces a minimal odor, does not require processing before use, and is more effective against mycobacteria.

Ethylene oxide is a type of alkylating agent that is used for gaseous sterilization. It is highly penetrating and can sterilize items within plastic bags such as catheters, disposable items in laboratories and clinical settings (like packaged Petri dishes), and other pieces of equipment. Ethylene oxide exposure is a form of cold sterilization, making it useful for the sterilization of heat-sensitive items. Great care needs to be taken with the use of ethylene oxide, however; it is carcinogenic, like the other alkylating agents, and is also highly explosive. With careful use and proper aeration of the products after treatment, ethylene oxide is highly effective, and ethylene oxide sterilizers are commonly found in medical settings for sterilizing packaged materials.

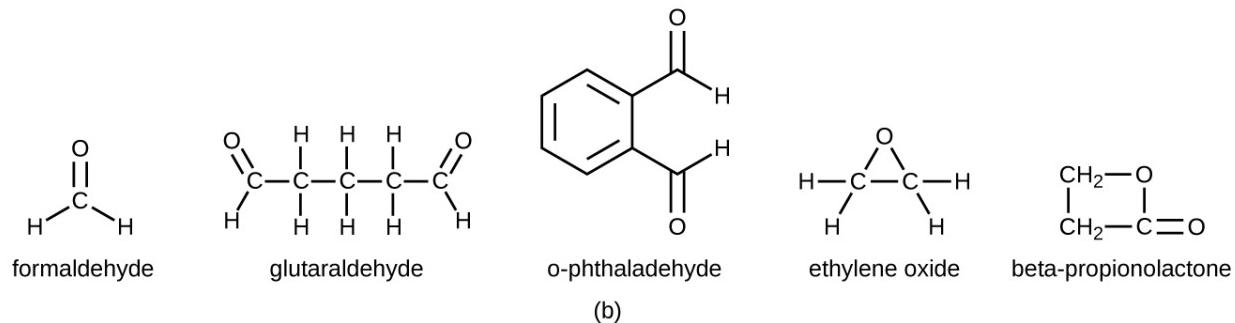
β -Propionolactone is an alkylating agent with a different chemical structure than the others already discussed. Like other alkylating agents, β -propionolactone binds to DNA, thereby inactivating it ([\[link\]](#)). It is a clear liquid with a strong odor and has the ability to kill endospores. As such, it has been used in either liquid form or as a vapor for the sterilization of medical instruments and tissue grafts, and it is a common component of vaccines, used to maintain their sterility. It has also been used for the sterilization of nutrient broth, as well as blood plasma, milk, and water. It is quickly metabolized by animals and humans to lactic acid. It is also an

irritant, however, and may lead to permanent damage of the eyes, kidneys, or liver. Additionally, it has been shown to be carcinogenic in animals; thus, precautions are necessary to minimize human exposure to β -propionolactone.[\[footnote\]](#)

Institute of Medicine. "Long-Term Health Effects of Participation in Project SHAD (Shipboard Hazard and Defense)." Washington, DC: The National Academies Press, 2007.



(a)



(b)

(a) Alkylating agents replace hydrogen atoms with alkyl groups. Here, guanine is alkylated, resulting in its hydrogen bonding with thymine, instead of cytosine. (b) The chemical structures of several alkylating agents.

Note:

- What chemical reaction do alkylating agents participate in?
- Why are alkylating agents not used as antiseptics?

Note:

Diehard Prions

Prions, the acellular, misfolded proteins responsible for incurable and fatal diseases such as kuru and Creutzfeldt-Jakob disease (see [Viroids, Virusoids, and Prions](#)), are notoriously difficult to destroy. Prions are extremely resistant to heat, chemicals, and radiation. They are also extremely infectious and deadly; thus, handling and disposing of prion-infected items requires extensive training and extreme caution.

Typical methods of disinfection can reduce but not eliminate the infectivity of prions. Autoclaving is not completely effective, nor are chemicals such as phenol, alcohols, formalin, and β -propiolactone. Even when fixed in formalin, affected brain and spinal cord tissues remain infectious.

Personnel who handle contaminated specimens or equipment or work with infected patients must wear a protective coat, face protection, and cut-resistant gloves. Any contact with skin must be immediately washed with detergent and warm water without scrubbing. The skin should then be washed with 1 N NaOH or a 1:10 dilution of bleach for 1 minute.

Contaminated waste must be incinerated or autoclaved in a strong basic solution, and instruments must be cleaned and soaked in a strong basic solution.

Note:

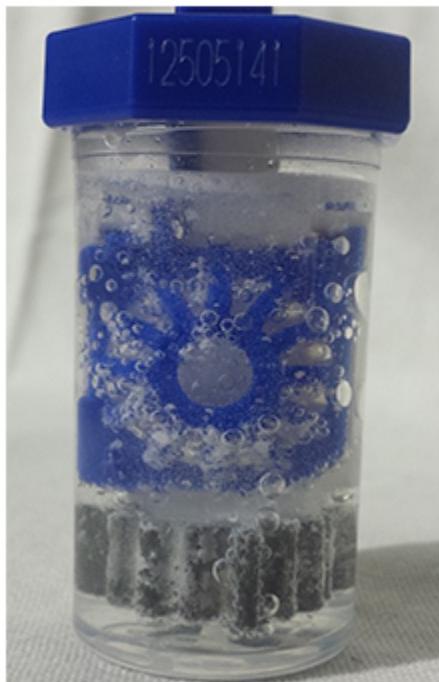


For more information on the handling of animals and prion-contaminated materials, visit the guidelines published on the [CDC](#) and [WHO](#) websites.

Peroxygens

Peroxygens are strong oxidizing agents that can be used as disinfectants or antiseptics. The most widely used **peroxygent** is hydrogen peroxide (H_2O_2), which is often used in solution to disinfect surfaces and may also be used as a gaseous agent. Hydrogen peroxide solutions are inexpensive skin antiseptics that break down into water and oxygen gas, both of which are environmentally safe. This decomposition is accelerated in the presence of light, so hydrogen peroxide solutions typically are sold in brown or opaque bottles. One disadvantage of using hydrogen peroxide as an antiseptic is that it also causes damage to skin that may delay healing or lead to scarring. Contact lens cleaners often include hydrogen peroxide as a disinfectant.

Hydrogen peroxide works by producing free radicals that damage cellular macromolecules. Hydrogen peroxide has broad-spectrum activity, working against gram-positive and gram-negative bacteria (with slightly greater efficacy against gram-positive bacteria), fungi, viruses, and endospores. However, bacteria that produce the oxygen-detoxifying enzymes catalase or peroxidase may have inherent tolerance to low hydrogen peroxide concentrations ([\[link\]](#)). To kill endospores, the length of exposure or concentration of solutions of hydrogen peroxide must be increased. Gaseous hydrogen peroxide has greater efficacy and can be used as a sterilant for rooms or equipment.



Catalase enzymatically converts highly reactive hydrogen peroxide (H_2O_2) into water and oxygen. Hydrogen peroxide can be used to clean wounds. Hydrogen peroxide is used to sterilize items such as contact lenses. (credit photos: modification of work by Kerry Ceszyk)

Plasma, a hot, ionized gas, described as the fourth state of matter, is useful for sterilizing equipment because it penetrates surfaces and kills vegetative cells and endospores. Hydrogen peroxide and peracetic acid, another commonly used peroxygen, each may be introduced as a plasma. Peracetic acid can be used as a liquid or plasma sterilant insofar as it readily kills endospores, is more effective than hydrogen peroxide even at rather low concentrations, and is immune to inactivation by catalases and peroxidases. It also breaks down to environmentally innocuous compounds; in this case, acetic acid and oxygen.

Other examples of peroxygens include benzoyl peroxide and carbamide peroxide. Benzoyl peroxide is a peroxygen that used in acne medication solutions. It kills the bacterium *Propionibacterium acnes*, which is associated with acne. Carbamide peroxide, an ingredient used in toothpaste, is a peroxygen that combats oral biofilms that cause tooth discoloration and halitosis (bad breath).[\[footnote\]](#) Last, ozone gas is a peroxygen with disinfectant qualities and is used to clean air or water supplies. Overall, peroxygens are highly effective and commonly used, with no associated environmental hazard.

Yao, C.S. et al. “In vitro antibacterial effect of carbamide peroxide on oral biofilm.” *Journal of Oral Microbiology* Jun 12, 2013.
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3682087/>. doi: 10.3402/jom.v5i0.20392.

Note:

- How do peroxides kill cells?

Chemical Food Preservatives

Chemical preservatives are used to inhibit microbial growth and minimize spoilage in some foods. Commonly used chemical preservatives include sorbic acid, benzoic acid, and propionic acid, and their more soluble salts potassium sorbate, sodium benzoate, and calcium propionate, all of which are used to control the growth of molds in acidic foods. Each of these preservatives is nontoxic and readily metabolized by humans. They are also flavorless, so they do not compromise the flavor of the foods they preserve.

Sorbic and benzoic acids exhibit increased efficacy as the pH decreases. Sorbic acid is thought to work by inhibiting various cellular enzymes, including those in the citric acid cycle, as well as catalases and peroxidases. It is added as a preservative in a wide variety of foods, including dairy, bread, fruit, and vegetable products. Benzoic acid is found naturally in

many types of fruits and berries, spices, and fermented products. It is thought to work by decreasing intracellular pH, interfering with mechanisms such as oxidative phosphorylation and the uptake of molecules such as amino acids into cells. Foods preserved with benzoic acid or sodium benzoate include fruit juices, jams, ice creams, pastries, soft drinks, chewing gum, and pickles.

Propionic acid is thought to both inhibit enzymes and decrease intracellular pH, working similarly to benzoic acid. However, propionic acid is a more effective preservative at a higher pH than either sorbic acid or benzoic acid. Propionic acid is naturally produced by some cheeses during their ripening and is added to other types of cheese and baked goods to prevent mold contamination. It is also added to raw dough to prevent contamination by the bacterium *Bacillus mesentericus*, which causes bread to become ropy.

Other commonly used chemical preservatives include sulfur dioxide and nitrites. Sulfur dioxide prevents browning of foods and is used for the preservation of dried fruits; it has been used in winemaking since ancient times. Sulfur dioxide gas dissolves in water readily, forming sulfites. Although sulfites can be metabolized by the body, some people have sulfite allergies, including asthmatic reactions. Additionally, sulfites degrade thiamine, an important nutrient in some foods. The mode of action of sulfites is not entirely clear, but they may interfere with the disulfide bond (see [[link](#)]) formation in proteins, inhibiting enzymatic activity. Alternatively, they may reduce the intracellular pH of the cell, interfering with proton motive force-driven mechanisms.

Nitrites are added to processed meats to maintain color and stop the germination of *Clostridium botulinum* endospores. Nitrites are reduced to nitric oxide, which reacts with heme groups and iron-sulfur groups. When nitric oxide reacts with the heme group within the myoglobin of meats, a red product forms, giving meat its red color. Alternatively, it is thought that when nitric acid reacts with the iron-sulfur enzyme ferredoxin within bacteria, this electron transport-chain carrier is destroyed, preventing ATP synthesis. Nitrosamines, however, are carcinogenic and can be produced through exposure of nitrite-preserved meats (e.g., hot dogs, lunch meat, breakfast sausage, bacon, meat in canned soups) to heat during cooking.

Natural Chemical Food Preservatives

The discovery of natural antimicrobial substances produced by other microbes has added to the arsenal of preservatives used in food. Nisin is an antimicrobial peptide produced by the bacterium *Lactococcus lactis* and is particularly effective against gram-positive organisms. Nisin works by disrupting cell wall production, leaving cells more prone to lysis. It is used to preserve cheeses, meats, and beverages.

Natamycin is an antifungal macrolide antibiotic produced by the bacterium *Streptomyces natalensis*. It was approved by the FDA in 1982 and is used to prevent fungal growth in various types of dairy products, including cottage cheese, sliced cheese, and shredded cheese. Natamycin is also used for meat preservation in countries outside the United States.

Note:

- What are the advantages and drawbacks of using sulfites and nitrites as food preservatives?

Key Concepts and Summary

- **Heavy metals**, including mercury, silver, copper, and zinc, have long been used for disinfection and preservation, although some have toxicity and environmental risks associated with them.
- **Halogens**, including chlorine, fluorine, and iodine, are also commonly used for disinfection. Chlorine compounds, including **sodium hypochlorite**, **chloramines**, and **chlorine dioxide**, are commonly used for water disinfection. Iodine, in both **tincture** and **iodophor** forms, is an effective antiseptic.
- **Alcohols**, including ethyl alcohol and isopropyl alcohol, are commonly used antiseptics that act by denaturing proteins and disrupting membranes.

- **Phenolics** are stable, long-acting disinfectants that denature proteins and disrupt membranes. They are commonly found in household cleaners, mouthwashes, and hospital disinfectants, and are also used to preserve harvested crops.
- The phenolic compound **triclosan**, found in antibacterial soaps, plastics, and textiles is technically an antibiotic because of its specific mode of action of inhibiting bacterial fatty-acid synthesis..
- **Surfactants**, including soaps and detergents, lower the surface tension of water to create emulsions that mechanically carry away microbes. Soaps are long-chain fatty acids, whereas detergents are synthetic surfactants.
- **Quaternary ammonium compounds (quats)** are cationic detergents that disrupt membranes. They are used in household cleaners, skin disinfectants, oral rinses, and mouthwashes.
- **Bisbiguanides** disrupt cell membranes, causing cell contents to gel. **Chlorhexidine** and **alexidine** are commonly used for surgical scrubs, for handwashing in clinical settings, and in prescription oral rinses.
- **Alkylating agents** effectively sterilize materials at low temperatures but are carcinogenic and may also irritate tissue. **Glutaraldehyde** and **o-phthalaldehyde** are used as hospital disinfectants but not as antiseptics. **Formaldehyde** is used for the storage of tissue specimens, as an embalming fluid, and in vaccine preparation to inactivate infectious agents. **Ethylene oxide** is a gas sterilant that can permeate heat-sensitive packaged materials, but it is also explosive and carcinogenic.
- **Peroxygens**, including **hydrogen peroxide**, **peracetic acid**, **benzoyl peroxide**, and ozone gas, are strong oxidizing agents that produce free radicals in cells, damaging their macromolecules. They are environmentally safe and are highly effective disinfectants and antiseptics.
- Chemical preservatives are added to a variety of foods. **Sorbic acid**, **benzoic acid**, **propionic acid**, and their more soluble salts inhibit enzymes or reduce intracellular pH.
- **Sulfites** are used in winemaking and food processing to prevent browning of foods.
- **Nitrites** are used to preserve meats and maintain color, but cooking nitrite-preserved meats may produce carcinogenic nitrosamines.

- **Nisin** and **natamycin** are naturally produced preservatives used in cheeses and meats. Nisin is effective against gram-positive bacteria and natamycin against fungi.

Short Answer

Exercise:

Problem:

Which solution of ethyl alcohol is more effective at inhibiting microbial growth: a 70% solution or a 100% solution? Why?

Exercise:

Problem:

When might a gas treatment be used to control microbial growth instead of autoclaving? What are some examples?

Exercise:

Problem:

What is the advantage of using an iodophor rather than iodine or an iodine tincture?

Critical Thinking

Exercise:

Problem:

Looking at [link] and reviewing the functional groups in [link], which alkylating agent shown lacks an aldehyde group?

Exercise:

Problem:

Do you think naturally produced antimicrobial products like nisin and natamycin should replace sorbic acid for food preservation? Why or why not?

Exercise:

Problem:

Why is the use of skin disinfecting compounds required for surgical scrubbing and not for everyday handwashing?

Testing the Effectiveness of Antiseptics and Disinfectants

LEARNING OBJECTIVES

- Describe why the phenol coefficient is used
- Compare and contrast the disk-diffusion, use-dilution, and in-use methods for testing the effectiveness of antiseptics, disinfectants, and sterilants

The effectiveness of various chemical disinfectants is reflected in the terms used to describe them. Chemical disinfectants are grouped by the power of their activity, with each category reflecting the types of microbes and viruses its component disinfectants are effective against. High-level germicides have the ability to kill vegetative cells, fungi, viruses, and endospores, leading to sterilization, with extended use.

Intermediate-level germicides, as their name suggests, are less effective against endospores and certain viruses, and low-level germicides kill only vegetative cells and certain enveloped viruses, and are ineffective against endospores.

However, several environmental conditions influence the potency of an antimicrobial agent and its effectiveness. For example, length of exposure is particularly important, with longer exposure increasing efficacy. Similarly, the concentration of the chemical agent is also important, with higher concentrations being more effective than lower ones. Temperature, pH, and other factors can also affect the potency of a disinfecting agent.

One method to determine the effectiveness of a chemical agent includes swabbing surfaces before and after use to confirm whether a sterile field was maintained during use. Additional

tests are described in the sections that follow. These tests allow for the maintenance of appropriate disinfection protocols in clinical settings, controlling microbial growth to protect patients, health-care workers, and the community.

Phenol Coefficient

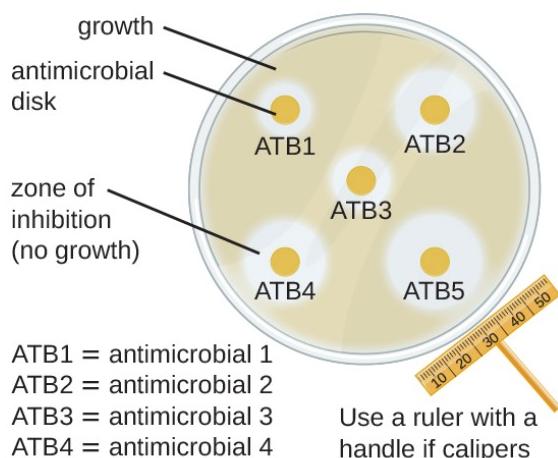
The effectiveness of a disinfectant or antiseptic can be determined in a number of ways. Historically, a chemical agent's effectiveness was often compared with that of phenol, the first chemical agent used by Joseph Lister. In 1903, British chemists Samuel Rideal (1863–1929) and J. T. Ainslie Walker (1868–1930) established a protocol to compare the effectiveness of a variety of chemicals with that of phenol, using as their test organisms *Staphylococcus aureus* (a gram-positive bacterium) and *Salmonella enterica* serovar Typhi (a gram-negative bacterium). They exposed the test bacteria to the antimicrobial chemical solutions diluted in water for 7.5 minutes. They then calculated a phenol coefficient for each chemical for each of the two bacteria tested. A **phenol coefficient** of 1.0 means that the chemical agent has about the same level of effectiveness as phenol. A chemical agent with a phenol coefficient of less than 1.0 is less effective than phenol. An example is formalin, with phenol coefficients of 0.3 (*S. aureus*) and 0.7 (*S. enterica* serovar Typhi). A chemical agent with a phenol coefficient greater than 1.0 is more effective than phenol, such as chloramine, with phenol coefficients of 133 and 100, respectively. Although the phenol coefficient was once a useful measure of effectiveness, it is no longer commonly used because the conditions and organisms used were arbitrarily chosen.

Note:

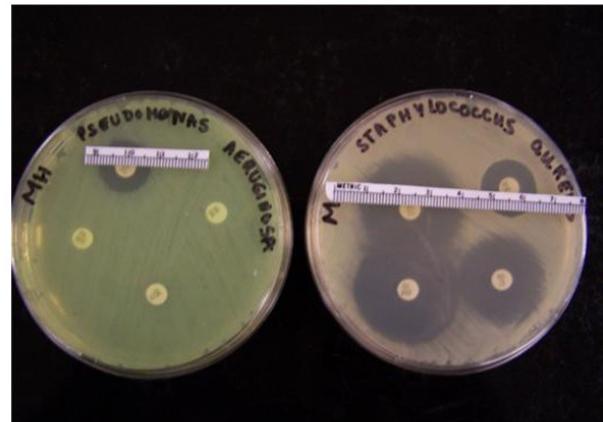
- What are the differences between the three levels of disinfectant effectiveness?

Disk-Diffusion Method

The **disk-diffusion method** involves applying different chemicals to separate, sterile filter paper disks ([\[link\]](#)). The disks are then placed on an agar plate that has been inoculated with the targeted bacterium and the chemicals diffuse out of the disks into the agar where the bacteria have been inoculated. As the “lawn” of bacteria grows, zones of inhibition of microbial growth are observed as clear areas around the disks. Although there are other factors that contribute to the sizes of zones of inhibition (e.g., whether the agent is water soluble and able to diffuse in the agar), larger zones typically correlate to increased inhibition effectiveness of the chemical agent. The diameter across each zone is measured in millimeters.



(a)



(b)

A disk-diffusion assay is used to determine the effectiveness of chemical agents against a particular microbe. (a) A plate is inoculated with various antimicrobial discs. The zone of inhibition around each disc indicates how effective that antimicrobial is against the particular species being tested. (b) On these plates, four antimicrobial agents are tested for efficacy in killing *Pseudomonas aeruginosa* (left) and

Staphylococcus aureus (right). These antimicrobials are much more effective at killing *S. aureus*, as indicated by the size of the zones of inhibition. (credit b: modification of work by American Society for Microbiology)

Note:

- When comparing the activities of two disinfectants against the same microbe, using the disk-diffusion assay, and assuming both are water soluble and can easily diffuse in the agar, would a more effective disinfectant have a larger zone of inhibition or a smaller one?

Use-Dilution Test

Other methods are also used for measuring the effectiveness of a chemical agent in clinical settings. The **use-dilution test** is commonly used to determine a chemical's disinfection effectiveness on an inanimate surface. For this test, a cylinder of stainless steel is dipped in a culture of the targeted microorganism and then dried. The cylinder is then dipped in solutions of disinfectant at various concentrations for a specified amount of time. Finally, the cylinder is transferred to a new test tube containing fresh sterile medium that does not contain disinfectant, and this test tube is incubated. Bacterial survival is demonstrated by the presence of turbidity in the medium, whereas killing of the target organism on the cylinder by the disinfectant will produce no turbidity.

The Association of Official Agricultural Chemists International (AOAC), a nonprofit group that establishes many protocol standards, has determined that a minimum of 59 of 60 replicates must show no growth in such a test to achieve a passing result, and the results must be repeatable from different batches of disinfectant and when performed on different days. Disinfectant

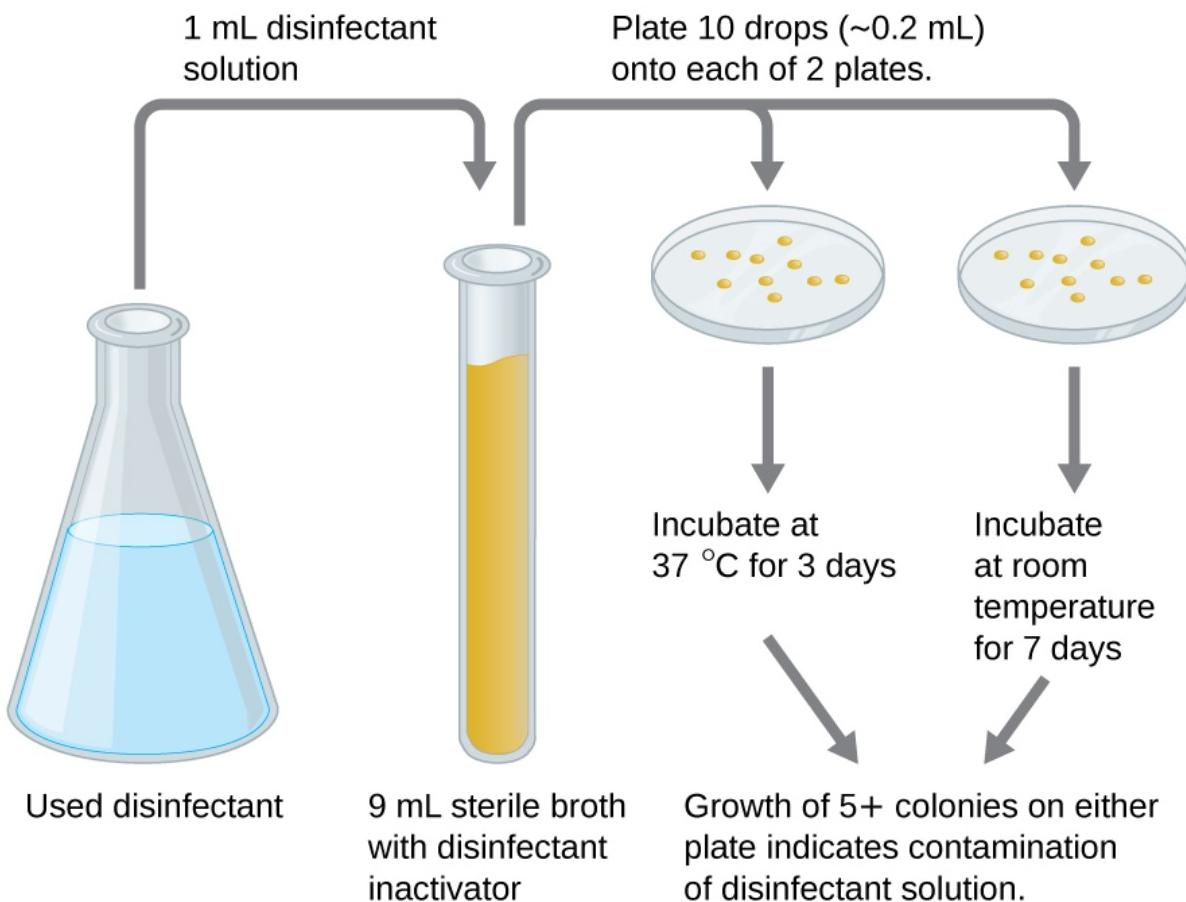
manufacturers perform use-dilution tests to validate the efficacy claims for their products, as designated by the EPA.

Note:

- Is the use-dilution test performed in a clinical setting? Why?

In-Use Test

An **in-use test** can determine whether an actively used solution of disinfectant in a clinical setting is microbially contaminated ([\[link\]](#)). A 1-mL sample of the used disinfectant is diluted into 9 mL of sterile broth medium that also contains a compound to inactivate the disinfectant. Ten drops, totaling approximately 0.2 mL of this mixture, are then inoculated onto each of two agar plates. One plate is incubated at 37 °C for 3 days and the other is incubated at room temperature for 7 days. The plates are monitored for growth of microbial colonies. Growth of five or more colonies on either plate suggests that viable microbial cells existed in the disinfectant solution and that it is contaminated. Such in-use tests monitor the effectiveness of disinfectants in the clinical setting.



Used disinfectant solutions in a clinical setting can be checked with the in-use test for contamination with microbes.

Note:

- What does a positive in-use test indicate?

Key Concepts and Summary

- Chemical disinfectants are grouped by the types of microbes and infectious agents they are effective against. **High-level germicides** kill vegetative cells, fungi, viruses, and endospores, and can ultimately lead to sterilization. **Intermediate-level germicides** cannot kill all viruses and are less effective against endospores. **Low-level germicides** kill vegetative cells and some enveloped viruses, but are ineffective against endospores.
- The effectiveness of a disinfectant is influenced by several factors, including length of exposure, concentration of disinfectant, temperature, and pH.
- Historically, the effectiveness of a chemical disinfectant was compared with that of phenol at killing *Staphylococcus aureus* and *Salmonella enterica* serovar Typhi, and a **phenol coefficient** was calculated.
- The **disk-diffusion method** is used to test the effectiveness of a chemical disinfectant against a particular microbe.
- The **use-dilution test** determines the effectiveness of a disinfectant on a surface. **In-use tests** can determine whether disinfectant solutions are being used correctly in clinical settings.

Critical Thinking

Exercise:

Problem:

What are some advantages of use-dilution and in-use tests compared with the disk-diffusion assay?

Antimicrobial Drugs - Introduction

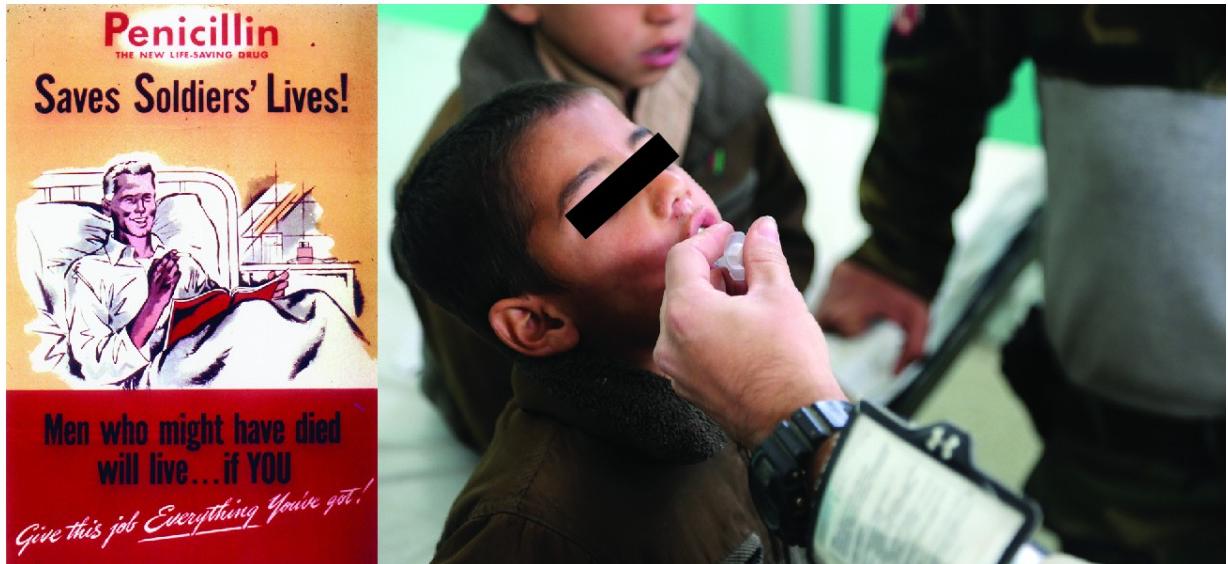
class="introduction"

First mass produced in the 1940s, penicillin was instrumental in saving millions of lives during World War II and was considered a wonder drug.

[footnote]

Today, overprescription of antibiotics (especially for childhood illnesses) has contributed to the evolution of drug-resistant pathogens.
(credit left: modification of work by Chemical Heritage Foundation; credit right: modification of work by U.S. Department of Defense)

“Treatment of
War Wounds: A
Historical
Review.”
Clinical
Orthopaedics
and Related
Research 467
no. 8
(2009):2168–
2191.



In nature, some microbes produce substances that inhibit or kill other microbes that might otherwise compete for the same resources. Humans have successfully exploited these abilities, using microbes to mass-produce substances that can be used as antimicrobial drugs. Since their discovery, antimicrobial drugs have saved countless lives, and they remain an essential tool for treating and controlling infectious disease. But their widespread and often unnecessary use has had an unintended side effect: the rise of multidrug-resistant microbial strains. In this chapter, we will discuss how antimicrobial drugs work, why microbes develop resistance, and what health professionals can do to encourage responsible use of antimicrobials.

History of Chemotherapy and Antimicrobial Discovery

LEARNING OBJECTIVES

- Compare and contrast natural, semisynthetic, and synthetic antimicrobial drugs
- Describe the chemotherapeutic approaches of ancient societies
- Describe the historically important individuals and events that led to the development of antimicrobial drugs

Use of Antimicrobials in Ancient Societies

Although the discovery of antimicrobials and their subsequent widespread use is commonly associated with modern medicine, there is evidence that humans have been exposed to antimicrobial compounds for millennia.

Chemical analyses of the skeletal remains of people from Nubia [[footnote](#)] (now found in present-day Sudan) dating from between 350 and 550 AD have shown residue of the antimicrobial agent tetracycline in high enough quantities to suggest the purposeful fermentation of tetracycline-producing *Streptomyces* during the beer-making process. The resulting beer, which was thick and gruel-like, was used to treat a variety of ailments in both adults and children, including gum disease and wounds. The antimicrobial properties of certain plants may also have been recognized by various cultures around the world, including Indian and Chinese herbalists ([\[link\]](#)) who have long used plants for a wide variety of medical purposes. Healers of many cultures understood the antimicrobial properties of fungi and their use of moldy bread or other mold-containing products to treat wounds has been well documented for centuries. [[footnote](#)] Today, while about 80% of the world's population still relies on plant-derived medicines, [[footnote](#)]

scientists are now discovering the active compounds conferring the medicinal benefits contained in many of these traditionally used plants.

M.L. Nelson et al. "Brief Communication: Mass Spectroscopic Characterization of Tetracycline in the Skeletal Remains of an Ancient Population from Sudanese Nubia 350–550 CE." *American Journal of Physical Anthropology* 143 no. 1 (2010):151–154.

M. Wainwright. "Moulds in Ancient and More Recent Medicine." *Mycologist* 3 no. 1 (1989):21–23.

S. Verma, S.P. Singh. "Current and Future Status of Herbal Medicines." *Veterinary World* 1 no. 11 (2008):347–350.



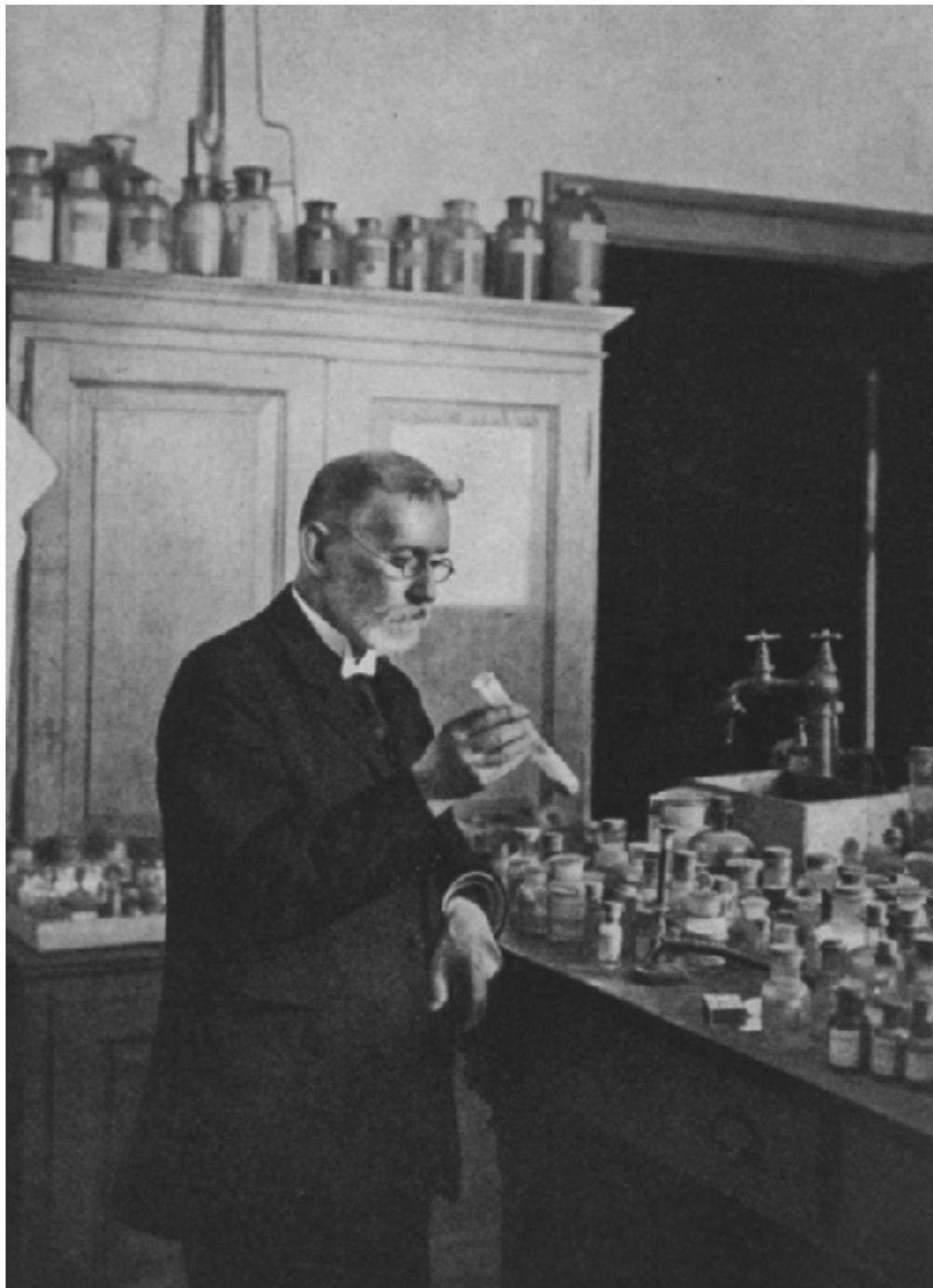
For millennia, Chinese herbalists have used many different species of plants for the treatment of a wide variety of human ailments.

Note:

- Give examples of how antimicrobials were used in ancient societies.

The First Antimicrobial Drugs

Societies relied on traditional medicine for thousands of years; however, the first half of the 20th century brought an era of strategic drug discovery. In the early 1900s, the German physician and scientist Paul Ehrlich (1854–1915) set out to discover or synthesize chemical compounds capable of killing infectious microbes without harming the patient. In 1909, after screening more than 600 arsenic-containing compounds, Ehrlich’s assistant Sahachiro Hata (1873–1938) found one such “magic bullet.” Compound 606 targeted the bacterium *Treponema pallidum*, the causative agent of syphilis. Compound 606 was found to successfully cure syphilis in rabbits and soon after was marketed under the name Salvarsan as a remedy for the disease in humans ([\[link\]](#)). Ehrlich’s innovative approach of systematically screening a wide variety of compounds remains a common strategy for the discovery of new antimicrobial agents even today.



Paul Ehrlich was influential in the discovery of Compound 606, an antimicrobial agent that proved to be an effective treatment for syphilis.

A few decades later, German scientists Josef Klarer, Fritz Mietzsch, and Gerhard Domagk discovered the antibacterial activity of a synthetic dye, prontosil, that could treat streptococcal and staphylococcal infections in mice. Domagk's own daughter was one of the first human recipients of the drug, which completely cured her of a severe streptococcal infection that had resulted from a poke with an embroidery needle. Gerhard Domagk (1895–1964) was awarded the Nobel Prize in Medicine in 1939 for his work with prontosil and sulfanilamide, the active breakdown product of prontosil in the body. Sulfanilamide, the first synthetic antimicrobial created, served as the foundation for the chemical development of a family of sulfa drugs. A **synthetic antimicrobial** is a drug that is developed from a chemical not found in nature. The success of the sulfa drugs led to the discovery and production of additional important classes of synthetic antimicrobials, including the quinolines and oxazolidinones.

A few years before the discovery of prontosil, scientist Alexander Fleming (1881–1955) made his own accidental discovery that turned out to be monumental. In 1928, Fleming returned from holiday and examined some old plates of staphylococci in his research laboratory at St. Mary's Hospital in London. He observed that contaminating mold growth (subsequently identified as a strain of *Penicillium notatum*) inhibited staphylococcal growth on one plate. Fleming, therefore, is credited with the discovery of **penicillin**, the first **natural antibiotic**, ([\[link\]](#)). Further experimentation showed that penicillin from the mold was antibacterial against streptococci, meningococci, and *Corynebacterium diphtheriae*, the causative agent of diphtheria.

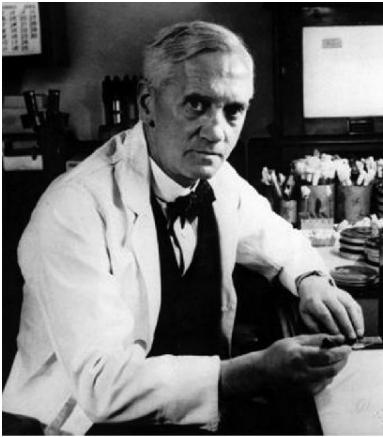
Fleming and his colleagues were credited with discovering and identifying penicillin, but its isolation and mass production were accomplished by a team of researchers at Oxford University under the direction of Howard Florey (1898–1968) and Ernst Chain (1906–1979) ([\[link\]](#)). In 1940, the research team purified penicillin and reported its success as an antimicrobial agent against streptococcal infections in mice. Their subsequent work with human subjects also showed penicillin to be very effective. Because of their important work, Fleming, Florey, and Chain were awarded the Nobel Prize in Physiology and Medicine in 1945.

In the early 1940s, scientist Dorothy Hodgkin (1910–1994), who studied crystallography at Oxford University, used X-rays to analyze the structure of a variety of natural products. In 1946, she determined the structure of penicillin, for which she was awarded the Nobel Prize in Chemistry in 1964. Once the structure was understood, scientists could modify it to produce a variety of semisynthetic penicillins. A **semisynthetic antimicrobial** is a chemically modified derivative of a natural antibiotic. The chemical modifications are generally designed to increase the range of bacteria targeted, increase stability, decrease toxicity, or confer other properties beneficial for treating infections.

Penicillin is only one example of a natural antibiotic. Also in the 1940s, Selman Waksman (1888–1973) ([\[link\]](#)), a prominent soil microbiologist at Rutgers University, led a research team that discovered several antimicrobials, including actinomycin, streptomycin, and neomycin. The discoveries of these antimicrobials stemmed from Waksman's study of fungi and the Actinobacteria, including soil bacteria in the genus *Streptomyces*, known for their natural production of a wide variety of antimicrobials. His work earned him the Nobel Prize in Physiology and Medicine in 1952. The actinomycetes are the source of more than half of all natural antibiotics [\[footnote\]](#) and continue to serve as an excellent reservoir for the discovery of novel antimicrobial agents. Some researchers argue that we have not yet come close to tapping the full antimicrobial potential of this group. [\[footnote\]](#)

J. Berdy. “Bioactive Microbial Metabolites.” *The Journal of Antibiotics* 58 no. 1 (2005):1–26.

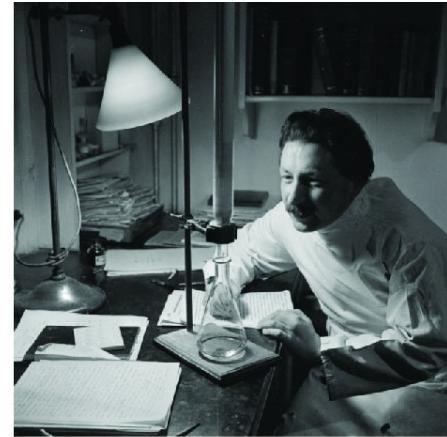
M. Baltz. “Antimicrobials from Actinomycetes: Back to the Future.” *Microbe* 2 no. 3 (2007):125–131.



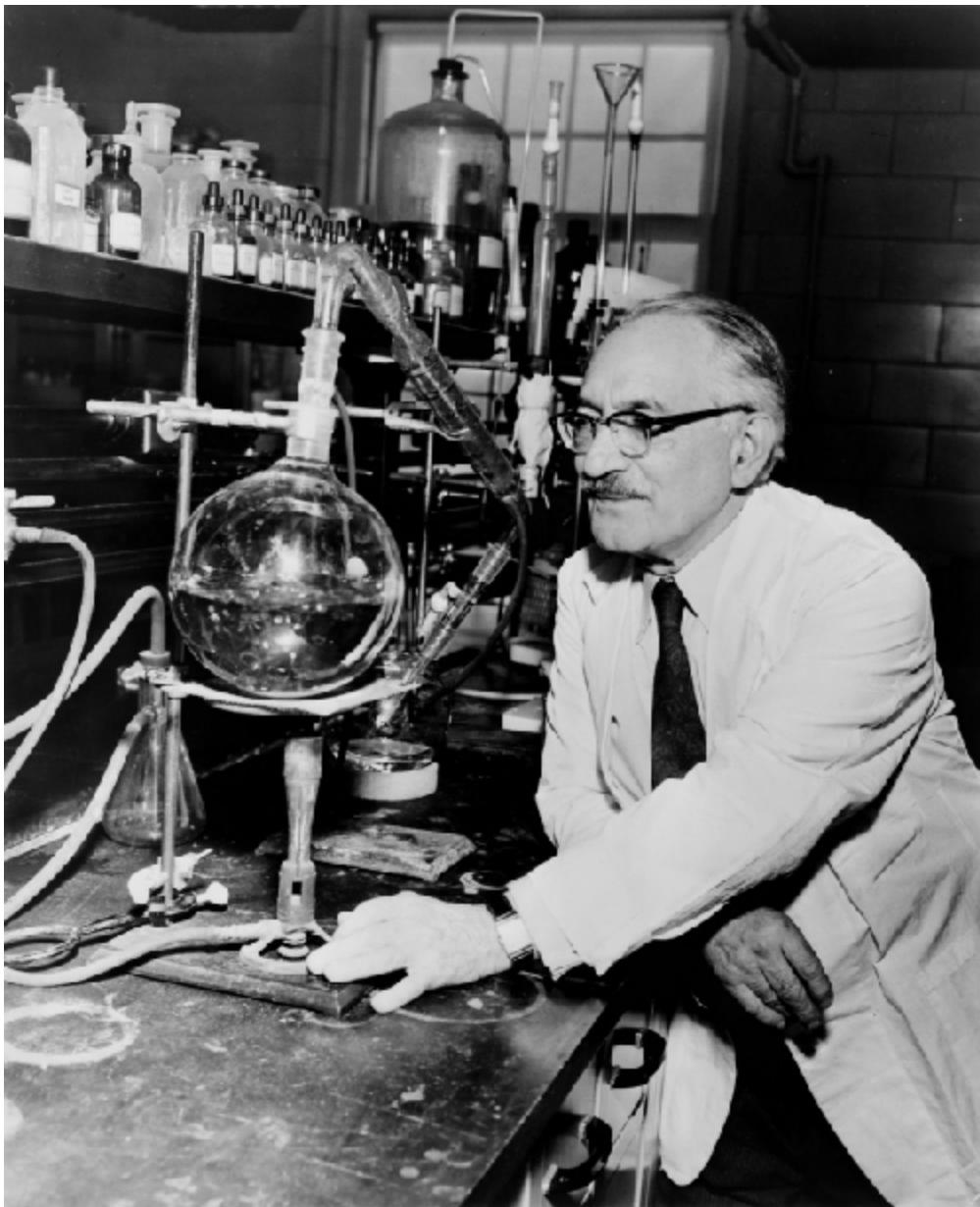
(a)



(b)



(a) Alexander Fleming was the first to discover a naturally produced antimicrobial, penicillin, in 1928. (b) Howard Florey and Ernst Chain discovered how to scale up penicillin production. Then they figured out how to purify it and showed its efficacy as an antimicrobial in animal and human trials in the early 1940s.



Selman Waksman was the first to show the vast antimicrobial production capabilities of a group of soil bacteria, the actinomycetes.

Note:

- Why is the soil a reservoir for antimicrobial resistance genes?

Key Concepts and Summary

- **Antimicrobial drugs** produced by purposeful fermentation and/or contained in plants have been used as traditional medicines in many cultures for millennia.
- The purposeful and systematic search for a chemical “magic bullet” that specifically target infectious microbes was initiated by Paul Ehrlich in the early 20th century.
- The discovery of the **natural antibiotic**, penicillin, by Alexander Fleming in 1928 started the modern age of antimicrobial discovery and research.
- Sulfanilamide, the first **synthetic antimicrobial**, was discovered by Gerhard Domagk and colleagues and is a breakdown product of the synthetic dye, prontosil.

Critical Thinking

Exercise:

Problem:

In nature, why do antimicrobial-producing microbes commonly also have antimicrobial resistance genes?

Fundamentals of Antimicrobial Chemotherapy

LEARNING OBJECTIVES

- Contrast bacteriostatic versus bactericidal antibacterial activities
- Contrast broad-spectrum drugs versus narrow-spectrum drugs
- Explain the significance of superinfections
- Discuss the significance of dosage and the route of administration of a drug
- Identify factors and variables that can influence the side effects of a drug
- Describe the significance of positive and negative interactions between drugs

Several factors are important in choosing the most appropriate antimicrobial drug therapy, including bacteriostatic versus bactericidal mechanisms, spectrum of activity, dosage and route of administration, the potential for side effects, and the potential interactions between drugs. The following discussion will focus primarily on antibacterial drugs, but the concepts translate to other antimicrobial classes.

Bacteriostatic Versus Bactericidal

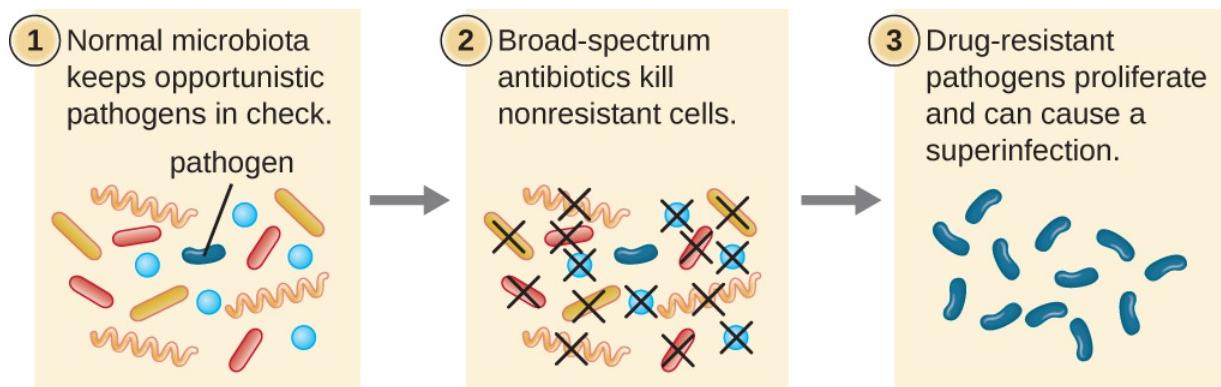
Antibacterial drugs can be either **bacteriostatic** or bactericidal in their interactions with target bacteria. Bacteriostatic drugs cause a reversible inhibition of growth, with bacterial growth restarting after elimination of the drug. By contrast, **bactericidal** drugs kill their target bacteria. The decision of whether to use a bacteriostatic or bactericidal drugs depends on

the type of infection and the immune status of the patient. In a patient with strong immune defenses, bacteriostatic and bactericidal drugs can be effective in achieving clinical cure. However, when a patient is immunocompromised, a bactericidal drug is essential for the successful treatment of infections. Regardless of the immune status of the patient, life-threatening infections such as acute endocarditis require the use of a bactericidal drug.

Spectrum of Activity

The spectrum of activity of an antibacterial drug relates to diversity of targeted bacteria. A **narrow-spectrum antimicrobial** targets only specific subsets of bacterial pathogens. For example, some narrow-spectrum drugs only target gram-positive bacteria, whereas others target only gram-negative bacteria. If the pathogen causing an infection has been identified, it is best to use a narrow-spectrum antimicrobial and minimize collateral damage to the normal microbiota. A **broad-spectrum antimicrobial** targets a wide variety of bacterial pathogens, including both gram-positive and gram-negative species, and is frequently used as empiric therapy to cover a wide range of potential pathogens while waiting on the laboratory identification of the infecting pathogen. Broad-spectrum antimicrobials are also used for polymicrobial infections (mixed infection with multiple bacterial species), or as prophylactic prevention of infections with surgery/invasive procedures. Finally, broad-spectrum antimicrobials may be selected to treat an infection when a narrow-spectrum drug fails because of development of drug resistance by the target pathogen.

The risk associated with using broad-spectrum antimicrobials is that they will also target a broad spectrum of the normal microbiota, increasing the risk of a **superinfection**, a secondary infection in a patient having a preexisting infection. A superinfection develops when the antibacterial intended for the preexisting infection kills the protective microbiota, allowing another pathogen resistant to the antibacterial to proliferate and cause a secondary infection ([\[link\]](#)). Common examples of superinfections that develop as a result of antimicrobial usage include yeast infections (candidiasis) and pseudomembranous colitis caused by *Clostridium difficile*, which can be fatal.



Broad-spectrum antimicrobial use may lead to the development of a superinfection. (credit: modification of work by Centers for Disease Control and Prevention)

Note:

- What is a superinfection and how does one arise?

Dosage and Route of Administration

The amount of medication given during a certain time interval is the **dosage**, and it must be determined carefully to ensure that optimum therapeutic drug levels are achieved at the site of infection without causing significant toxicity (side effects) to the patient. Each drug class is associated with a variety of potential side effects, and some of these are described for specific drugs later in this chapter. Despite best efforts to optimize dosing, allergic reactions and other potentially serious side effects do occur. Therefore, the goal is to select the optimum dosage that will minimize the risk of side effects while still achieving clinical cure, and there are important factors to consider when selecting the best dose and dosage interval. For example, in children, dose is based upon the patient's mass.

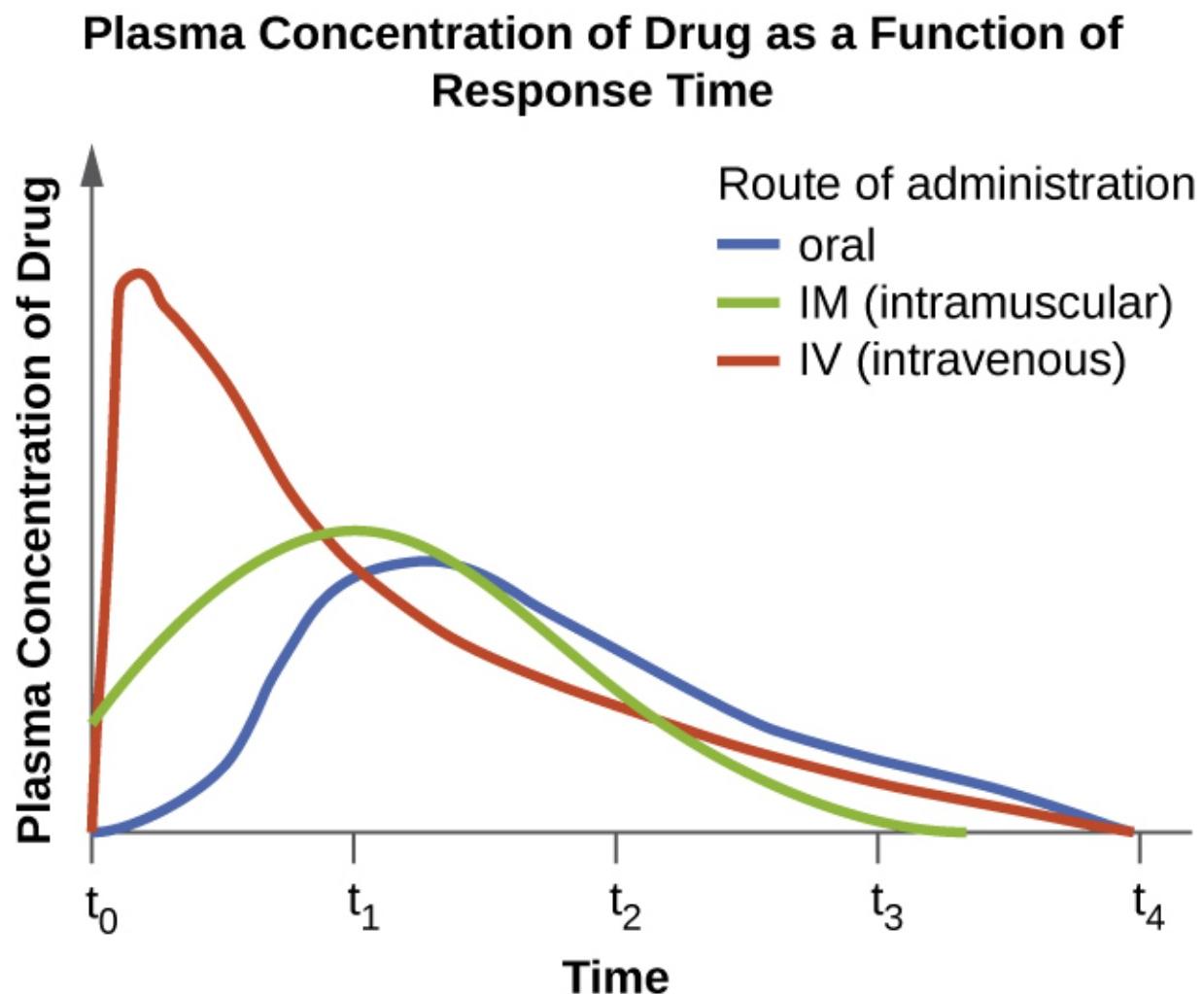
However, the same is not true for adults and children 12 years of age and older, for which there is typically a single standard dose regardless of the patient's mass. With the great variability in adult body mass, some experts have argued that mass should be considered for all patients when determining appropriate dosage.[\[footnote\]](#) An additional consideration is how drugs are metabolized and eliminated from the body. In general, patients with a history of liver or kidney dysfunction may experience reduced drug metabolism or clearance from the body, resulting in increased drug levels that may lead to toxicity and make them more prone to side effects.

M.E. Falagas, D.E. Karageorgopoulos. "Adjustment of Dosing of Antimicrobial Agents for Bodyweight in Adults." *The Lancet* 375 no. 9710 (2010):248–251.

There are also some factors specific to the drugs themselves that influence appropriate dose and time interval between doses. For example, the half-life, or rate at which 50% of a drug is eliminated from the plasma, can vary significantly between drugs. Some drugs have a short half-life of only 1 hour and must be given multiple times a day, whereas other drugs have half-lives exceeding 12 hours and can be given as a single dose every 24 hours. Although a longer half-life can be considered an advantage for an antibacterial when it comes to convenient dosing intervals, the longer half-life can also be a concern for a drug that has serious side effects because drug levels may remain toxic for a longer time. Last, some drugs are dose dependent, meaning they are more effective when administered in large doses to provide high levels for a short time at the site of infection. Others are time dependent, meaning they are more effective when lower optimum levels are maintained over a longer period of time.

The **route of administration**, the method used to introduce a drug into the body, is also an important consideration for drug therapy. Drugs that can be administered orally are generally preferred because patients can more conveniently take these drugs at home. However, some drugs are not absorbed easily from the gastrointestinal (GI) tract into the bloodstream. These drugs are often useful for treating diseases of the intestinal tract, such as tapeworms treated with niclosamide, or for decontaminating the bowel, as with colistin. Some drugs that are not absorbed easily, such as bacitracin,

polymyxin, and several antifungals, are available as topical preparations for treatment of superficial skin infections. Sometimes, patients may not initially be able to take oral medications because of their illness (e.g., vomiting, intubation for respirator). When this occurs, and when a chosen drug is not absorbed in the GI tract, administration of the drug by a parenteral route (intravenous or intramuscular injection) is preferred and typically is performed in health-care settings. For most drugs, the plasma levels achieved by intravenous administration is substantially higher than levels achieved by oral or intramuscular administration, and this can also be an important consideration when choosing the route of administration for treating an infection ([\[link\]](#)).



On this graph, t_0 represents the time at which a drug dose is administered. The curves illustrate how plasma concentration of the drug changes over specific intervals of time (t_1 through t_4). As the graph shows, when a drug is administered intravenously, the concentration peaks very quickly and then gradually decreases. When drugs are administered orally or intramuscularly, it takes longer for the concentration to reach its peak.

Note:

- List five factors to consider when determining the dosage of a drug.
- Name some typical side effects associated with drugs and identify some factors that might contribute to these side effects.

Drug Interactions

For the optimum treatment of some infections, two antibacterial drugs may be administered together to provide a synergistic interaction that is better than the efficacy of either drug alone. A classic example of synergistic combinations is trimethoprim and sulfamethoxazole (Bactrim).

Individually, these two drugs provide only bacteriostatic inhibition of bacterial growth, but combined, the drugs are bactericidal.

Whereas synergistic drug interactions provide a benefit to the patient, antagonistic interactions produce harmful effects. Antagonism can occur between two antimicrobials or between antimicrobials and nonantimicrobials being used to treat other conditions. The effects vary depending on the drugs involved, but antagonistic interactions may cause loss of drug activity, decreased therapeutic levels due to increased metabolism and elimination, or increased potential for toxicity due to decreased metabolism and elimination. As an example, some antibacterials

are absorbed most effectively from the acidic environment of the stomach. If a patient takes antacids, however, this increases the pH of the stomach and negatively impacts the absorption of these antimicrobials, decreasing their effectiveness in treating an infection. Studies have also shown an association between use of some antimicrobials and failure of oral contraceptives.[\[footnote\]](#)

B.D. Dickinson et al. “Drug Interactions between Oral Contraceptives and Antibiotics.” *Obstetrics & Gynecology* 98, no. 5 (2001):853–860.

Note:

- Explain the difference between synergistic and antagonistic drug interactions.

Note:

Resistance Police

In the United States and many other countries, most antimicrobial drugs are self-administered by patients at home. Unfortunately, many patients stop taking antimicrobials once their symptoms dissipate and they feel better. If a 10-day course of treatment is prescribed, many patients only take the drug for 5 or 6 days, unaware of the negative consequences of not completing the full course of treatment. A shorter course of treatment not only fails to kill the target organisms to expected levels, it also selects for drug-resistant variants within the target population and within the patient’s microbiota.

Patients’ nonadherence especially amplifies drug resistance when the recommended course of treatment is long. Treatment for tuberculosis (TB) is a case in point, with the recommended treatment lasting from 6 months to a year. The CDC estimates that about one-third of the world’s population is infected with TB, most living in underdeveloped or underserved regions where antimicrobial drugs are available over the counter. In such countries, there may be even lower rates of adherence than in developed areas.

Nonadherence leads to antibiotic resistance and more difficulty in

controlling pathogens. As a direct result, the emergence of multidrug-resistant and extensively drug-resistant strains of TB is becoming a huge problem.

Overprescription of antimicrobials also contributes to antibiotic resistance. Patients often demand antibiotics for diseases that do not require them, like viral colds and ear infections. Pharmaceutical companies aggressively market drugs to physicians and clinics, making it easy for them to give free samples to patients, and some pharmacies even offer certain antibiotics free to low-income patients with a prescription.

In recent years, various initiatives have aimed to educate parents and clinicians about the judicious use of antibiotics. However, a recent study showed that, between 2000 and 2013, the parental expectation for antimicrobial prescriptions for children actually increased ([\[link\]](#)).

One possible solution is a regimen called directly observed therapy (DOT), which involves the supervised administration of medications to patients.

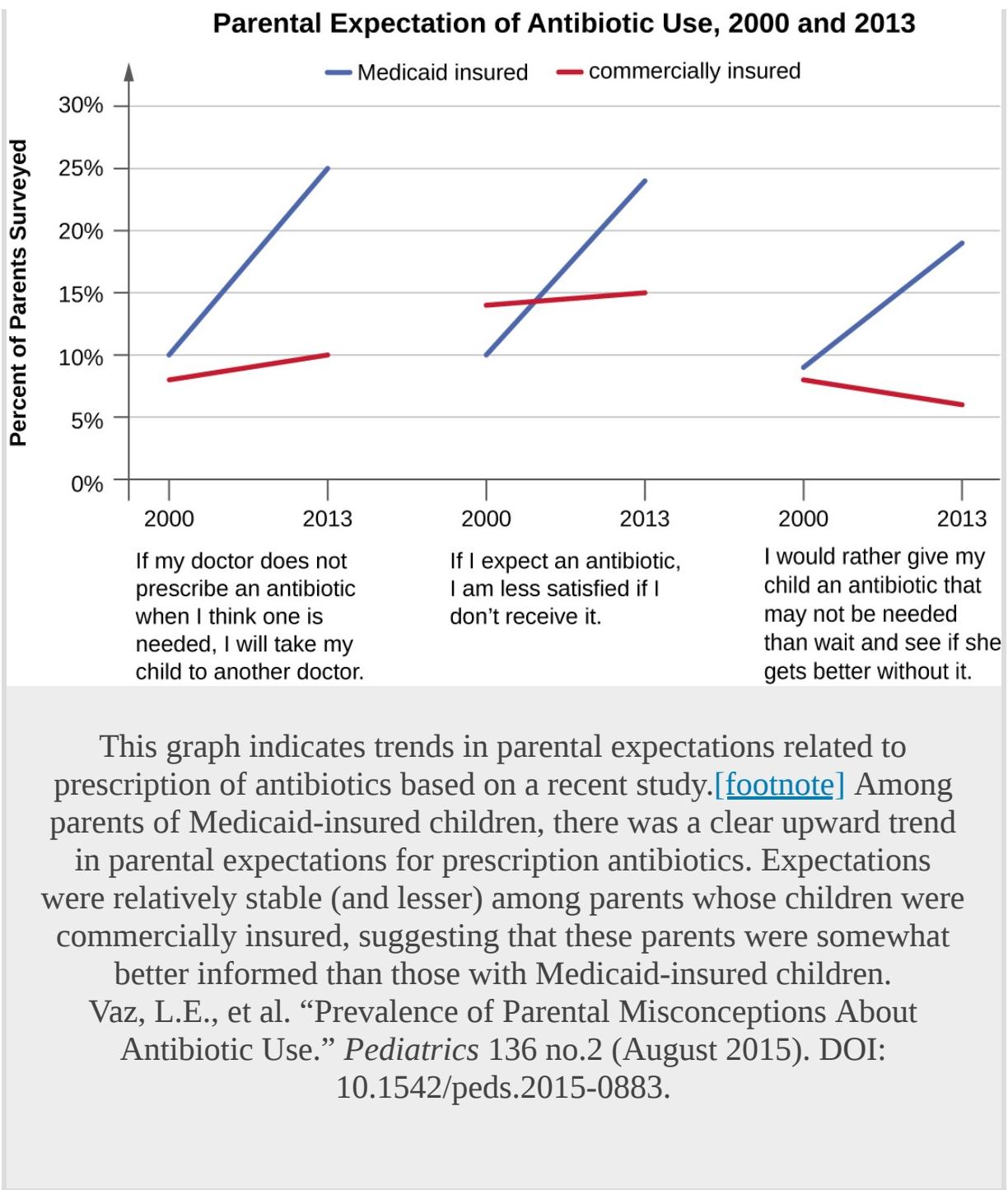
Patients are either required to visit a health-care facility to receive their medications, or health-care providers must administer medication in patients' homes or another designated location. DOT has been implemented in many cases for the treatment of TB and has been shown to be effective; indeed, DOT is an integral part of WHO's global strategy for eradicating TB.[\[footnote\]](#),[\[footnote\]](#) But is this a practical strategy for all antibiotics? Would patients taking penicillin, for example, be more or less likely to adhere to the full course of treatment if they had to travel to a health-care facility for each dose? And who would pay for the increased cost associated with DOT? When it comes to overprescription, should someone be policing physicians or drug companies to enforce best practices? What group should assume this responsibility, and what penalties would be effective in discouraging overprescription?

Centers for Disease Control and Prevention. “Tuberculosis (TB).”

<http://www.cdc.gov/tb/education/ssmodules/module9/ss9reading2.htm>.

Accessed June 2, 2016.

World Health Organization. “Tuberculosis (TB): The Five Elements of DOTS.” <http://www.who.int/tb/dots/whatisdots/en/>. Accessed June 2, 2016.



Key Concepts and Summary

- Antimicrobial drugs can be **bacteriostatic** or **bactericidal**, and these characteristics are important considerations when selecting the most appropriate drug.
- The use of **narrow-spectrum** antimicrobial drugs is preferred in many cases to avoid **superinfection** and the development of antimicrobial resistance.
- **Broad-spectrum** antimicrobial use is warranted for serious systemic infections when there is no time to determine the causative agent, when narrow-spectrum antimicrobials fail, or for the treatment or prevention of infections with multiple types of microbes.
- The **dosage** and **route of administration** are important considerations when selecting an antimicrobial to treat an infection. Other considerations include the patient's age, mass, ability to take oral medications, liver and kidney function, and possible interactions with other drugs the patient may be taking.

Critical Thinking

Exercise:

Problem:

Why are yeast infections a common type of superinfection that results from long-term use of broad-spectrum antimicrobials?

Exercise:

Problem:

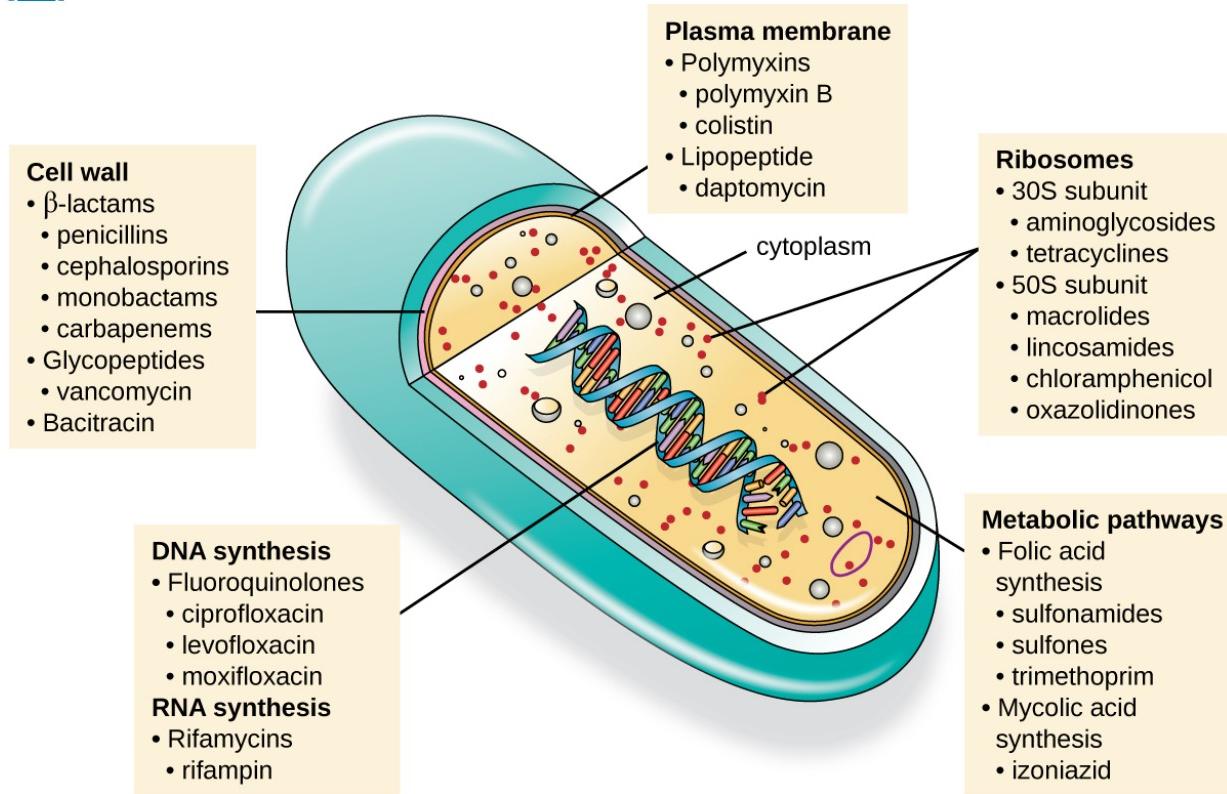
Too often patients will stop taking antimicrobial drugs before the prescription is finished. What are factors that cause a patient to stop too soon, and what negative impacts could this have?

Mechanisms of Antibacterial Drugs

LEARNING OBJECTIVE

- Describe the mechanisms of action associated with drugs that inhibit cell wall biosynthesis, protein synthesis, membrane function, nucleic acid synthesis, and metabolic pathways

An important quality for an antimicrobial drug is **selective toxicity**, meaning that it selectively kills or inhibits the growth of microbial targets while causing minimal or no harm to the host. Most antimicrobial drugs currently in clinical use are antibacterial because the prokaryotic cell provides a greater variety of unique targets for selective toxicity, in comparison to fungi, parasites, and viruses. Each class of antibacterial drugs has a unique **mode of action** (the way in which a drug affects microbes at the cellular level), and these are summarized in [\[link\]](#) and [\[link\]](#).



There are several classes of antibacterial compounds that are typically classified based on their bacterial target.

Common Antibacterial Drugs by Mode of Action		
Mode of Action	Target	Drug Class
Inhibit cell wall biosynthesis	Penicillin-binding proteins	β -lactams: penicillins, cephalosporins, monobactams, carbapenems
	Peptidoglycan subunits	Glycopeptides
	Peptidoglycan subunit transport	Bacitracin
Inhibit biosynthesis of proteins	30S ribosomal subunit	Aminoglycosides, tetracyclines
	50S ribosomal subunit	Macrolides, lincosamides, chloramphenicol, oxazolidinones
Disrupt membranes	Lipopolysaccharide, inner and outer membranes	Polymyxin B, colistin, daptomycin
Inhibit nucleic acid synthesis	RNA	Rifamycin
	DNA	Fluoroquinolones
Antimetabolites	Folic acid synthesis enzyme	Sulfonamides, trimethoprim
	Mycolic acid synthesis enzyme	Isonicotinic acid hydrazide
Mycobacterial adenosine triphosphate (ATP) synthase inhibitor	Mycobacterial ATP synthase	Diarylquinoline

Inhibitors of Cell Wall Biosynthesis

Several different classes of antibiotics block steps in the biosynthesis of peptidoglycan, making cells more susceptible to osmotic lysis ([\[link\]](#)). Therefore, antibiotics that target cell wall biosynthesis are bactericidal in their action. Because human cells do not make peptidoglycan, this mode of action is an excellent example of selective toxicity.

Penicillin, the first antibiotic discovered, is one of several antibiotics within a class called **β -lactams**. This group of compounds includes the penicillins, cephalosporins, monobactams, and carbapenems, and is characterized by the presence of a β -lactam ring found within the central structure of the drug molecule ([\[link\]](#)). The β -lactam antibiotics block the crosslinking of peptide chains during the biosynthesis of new peptidoglycan in the bacterial cell wall. They are able to block this process because the β -lactam structure is similar to the structure of the peptidoglycan subunit component that is recognized by the crosslinking transpeptidase enzyme, also known as a penicillin-binding protein (PBP). Although the β -lactam ring must remain unchanged for these drugs to retain their antibacterial activity, strategic chemical changes to the R groups have allowed for development of a wide variety of semisynthetic β -lactam drugs with increased potency, expanded spectrum of activity, and longer half-lives for better dosing, among other characteristics.

Penicillin G and penicillin V are natural antibiotics from fungi and are primarily active against gram-positive bacterial pathogens, and a few gram-negative bacterial pathogens such as *Pasteurella multocida*. [\[link\]](#)

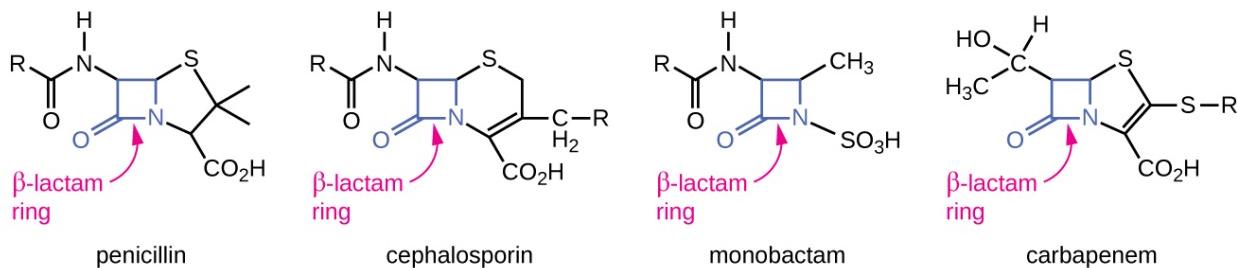
summarizes the semisynthetic development of some of the penicillins. Adding an amino group (-NH₂) to penicillin G created the aminopenicillins (i.e., ampicillin and amoxicillin) that have increased spectrum of activity against more gram-negative pathogens. Furthermore, the addition of a hydroxyl group (-OH) to amoxicillin increased acid stability, which allows for improved oral absorption. Methicillin is a semisynthetic penicillin that was developed to address the spread of enzymes (penicillinases) that were inactivating the other penicillins. Changing the R group of penicillin G to the more bulky dimethoxyphenyl group provided protection of the β-lactam ring from enzymatic destruction by penicillinases, giving us the first penicillinase-resistant penicillin.

Similar to the penicillins, **cephalosporins** contain a β-lactam ring ([\[link\]](#)) and block the transpeptidase activity of penicillin-binding proteins. However, the β-lactam ring of cephalosporins is fused to a six-member ring, rather than the five-member ring found in penicillins. This chemical difference provides cephalosporins with an increased resistance to enzymatic inactivation by **β-lactamases**. The drug cephalosporin C was originally isolated from the fungus *Cephalosporium acremonium* in the 1950s and has a similar spectrum of activity to that of penicillin against gram-positive bacteria but is active against more gram-negative bacteria than penicillin. Another important structural difference is that cephalosporin C possesses two R groups, compared with just one R group for penicillin, and this provides for greater diversity in chemical alterations and development of semisynthetic cephalosporins. The family of semisynthetic cephalosporins is much larger than the penicillins, and these drugs have been classified into generations based primarily on their spectrum of activity, increasing in spectrum from the narrow-spectrum, first-generation cephalosporins to the broad-spectrum, fourth-generation cephalosporins. A new fifth-generation cephalosporin has been developed that is active against methicillin-resistant *Staphylococcus aureus* (MRSA).

The carbapenems and monobactams also have a β-lactam ring as part of their core structure, and they inhibit the transpeptidase activity of penicillin-binding proteins. The only monobactam used clinically is aztreonam. It is a narrow-spectrum antibacterial with activity only against gram-negative bacteria. In contrast, the carbapenem family includes a variety of semisynthetic drugs (imipenem, meropenem, and doripenem) that provide very broad-spectrum activity against gram-positive and gram-negative bacterial pathogens.

The drug **vancomycin**, a member of a class of compounds called the **glycopeptides**, was discovered in the 1950s as a natural antibiotic from the actinomycete *Amycolatopsis orientalis*. Similar to the β-lactams, vancomycin inhibits cell wall biosynthesis and is bactericidal. However, in contrast to the β-lactams, the structure of vancomycin is not similar to that of cell-wall peptidoglycan subunits and does not directly inactivate penicillin-binding proteins. Rather, vancomycin is a very large, complex molecule that binds to the end of the peptide chain of cell wall precursors, creating a structural blockage that prevents the cell wall subunits from being incorporated into the growing N-acetylglucosamine and N-acetylmuramic acid (NAM-NAG) backbone of the peptidoglycan structure (transglycosylation). Vancomycin also structurally blocks transpeptidation. Vancomycin is bactericidal against gram-positive bacterial pathogens, but it is not active against gram-negative bacteria because of its inability to penetrate the protective outer membrane.

The drug **bacitracin** consists of a group of structurally similar peptide antibiotics originally isolated from *Bacillus subtilis*. Bacitracin blocks the activity of a specific cell-membrane molecule that is responsible for the movement of peptidoglycan precursors from the cytoplasm to the exterior of the cell, ultimately preventing their incorporation into the cell wall. Bacitracin is effective against a wide range of bacteria, including gram-positive organisms found on the skin, such as *Staphylococcus* and *Streptococcus*. Although it may be administered orally or intramuscularly in some circumstances, bacitracin has been shown to be nephrotoxic (damaging to the kidneys). Therefore, it is more commonly combined with neomycin and polymyxin in topical ointments such as Neosporin.



R group	$-\text{CH}_2-\text{C}_6\text{H}_5$	$\text{CH}_2-\text{O}-\text{C}_6\text{H}_5$	$-\text{CH}(\text{NH}_2)-\text{C}_6\text{H}_5$	$-\text{CH}(\text{NH}_2)-\text{C}_6\text{H}_4-\text{OH}$	$-\text{CH}_2-\text{C}_6\text{H}_3(\text{CH}_3\text{O})_2$
Drug name	penicillin G	penicillin V	ampicillin	amoxicillin	methicillin
Spectrum of activity	G+ and a few G-	similar to penicillin G	G+ and more G- than penicillin	similar to ampicillin	G+ only, including beta-lactamase producers
Route of administration	parenteral	oral	parenteral and oral	oral (better than ampicillin)	parenteral

Penicillins, cephalosporins, monobactams, and carbapenems all contain a beta-lactam ring, the site of attack by inactivating beta-lactamase enzymes. Although they all share the same nucleus, various penicillins differ from each other in the structure of their R groups. Chemical changes to the R groups provided increased spectrum of activity, acid stability, and resistance to beta-lactamase degradation.

Drugs that Inhibit Bacterial Cell Wall Synthesis

Mechanism of Action	Drug Class	Specific Drugs	Natural or Semisynthetic	Spectrum of Activity
Interact directly with PBPs and inhibit transpeptidase activity	Penicillins	Penicillin G, penicillin V	Natural	Narrow-spectrum against gram-positive and a few gram-negative bacteria

Drugs that Inhibit Bacterial Cell Wall Synthesis				
Mechanism of Action	Drug Class	Specific Drugs	Natural or Semisynthetic	Spectrum of Activity
Inhibition of penicillin-binding proteins (PBPs)	Penicillins	Ampicillin, amoxicillin	Semisynthetic	Narrow-spectrum against gram-positive bacteria but with increased gram-negative spectrum
		Methicillin	Semisynthetic	Narrow-spectrum against gram-positive bacteria only, including strains producing penicillinase
	Cephalosporins	Cephalosporin C	Natural	Narrow-spectrum similar to penicillin but with increased gram-negative spectrum
	First-generation cephalosporins	First-generation cephalosporins	Semisynthetic	Narrow-spectrum similar to cephalosporin C
		Second-generation cephalosporins	Semisynthetic	Narrow-spectrum but with increased gram-negative spectrum compared with first generation

Drugs that Inhibit Bacterial Cell Wall Synthesis				
Mechanism of Action	Drug Class	Specific Drugs	Natural or Semisynthetic	Spectrum of Activity
		Third- and fourth-generation cephalosporins	Semisynthetic	Broad-spectrum against gram-positive and gram-negative bacteria, including some β -lactamase producers
		Fifth-generation cephalosporins	Semisynthetic	Broad-spectrum against gram-positive and gram-negative bacteria, including MRSA
	Monobactams	Aztreonam	Semisynthetic	Narrow-spectrum against gram-negative bacteria, including some β -lactamase producers
	Carbapenems	Imipenem, meropenem, doripenem	Semisynthetic	Broadest spectrum of the β -lactams against gram-positive and gram-negative bacteria, including many β -lactamase producers

Drugs that Inhibit Bacterial Cell Wall Synthesis				
Mechanism of Action	Drug Class	Specific Drugs	Natural or Semisynthetic	Spectrum of Activity
Large molecules that bind to the peptide chain of peptidoglycan subunits, blocking transglycosylation and transpeptidation	Glycopeptides	Vancomycin	Natural	Narrow spectrum against gram-positive bacteria only, including multidrug-resistant strains
Block transport of peptidoglycan subunits across cytoplasmic membrane	Bacitracin	Bacitracin	Natural	Broad-spectrum against gram-positive and gram-negative bacteria

Note:

- Describe the mode of action of β -lactams.

Inhibitors of Protein Biosynthesis

The cytoplasmic ribosomes found in animal cells (80S) are structurally distinct from those found in bacterial cells (70S), making protein biosynthesis a good selective target for antibacterial drugs. Several types of protein biosynthesis inhibitors are discussed in this section and are summarized in [\[link\]](#).

Protein Synthesis Inhibitors That Bind the 30S Subunit

Aminoglycosides are large, highly polar antibacterial drugs that bind to the 30S subunit of bacterial ribosomes, impairing the proofreading ability of the ribosomal complex. This impairment causes mismatches between codons and anticodons, resulting in the production of proteins with incorrect amino acids and shortened proteins that insert into the cytoplasmic membrane. Disruption of the cytoplasmic membrane by the faulty proteins kills the bacterial cells. The **aminoglycosides**, which include drugs such as streptomycin, gentamicin, neomycin, and kanamycin, are potent broad-spectrum antibiotics. However, aminoglycosides have been shown to be nephrotoxic (damaging to kidney), neurotoxic (damaging to the nervous system), and ototoxic (damaging to the ear).

Another class of antibacterial compounds that bind to the 30S subunit is the **tetracyclines**. In contrast to aminoglycosides, these drugs are bacteriostatic and inhibit protein synthesis by blocking the association of tRNAs with the ribosome during translation. Naturally occurring tetracyclines produced by various strains of *Streptomyces* were first discovered in the 1940s, and several semisynthetic tetracyclines, including doxycycline and tigecycline have also been produced. Although the tetracyclines are broad spectrum in their coverage of bacterial pathogens,

side effects that can limit their use include phototoxicity, permanent discoloration of developing teeth, and liver toxicity with high doses or in patients with kidney impairment.

Protein Synthesis Inhibitors That Bind the 50S Subunit

There are several classes of antibacterial drugs that work through binding to the 50S subunit of bacterial ribosomes. The macrolide antibacterial drugs have a large, complex ring structure and are part of a larger class of naturally produced secondary metabolites called polyketides, complex compounds produced in a stepwise fashion through the repeated addition of two-carbon units by a mechanism similar to that used for fatty acid synthesis. Macrolides are broad-spectrum, bacteriostatic drugs that block elongation of proteins by inhibiting peptide bond formation between specific combinations of amino acids. The first macrolide was **erythromycin**. It was isolated in 1952 from *Streptomyces erythreus* and prevents translocation. Semisynthetic macrolides include azithromycin and telithromycin. Compared with erythromycin, **azithromycin** has a broader spectrum of activity, fewer side effects, and a significantly longer half-life (1.5 hours for erythromycin versus 68 hours for azithromycin) that allows for once-daily dosing and a short 3-day course of therapy (i.e., Zpac formulation) for most infections. Telithromycin is the first semisynthetic within the class known as ketolides. Although telithromycin shows increased potency and activity against macrolide-resistant pathogens, the US Food and Drug Administration (FDA) has limited its use to treatment of community-acquired pneumonia and requires the strongest “black box warning” label for the drug because of serious hepatotoxicity.

The **lincosamides** include the naturally produced **lincomycin** and semisynthetic **clindamycin**. Although structurally distinct from macrolides, lincosamides are similar in their mode of action to the macrolides through binding to the 50S ribosomal subunit and preventing peptide bond formation. Lincosamides are particularly active against streptococcal and staphylococcal infections.

The drug **chloramphenicol** represents yet another structurally distinct class of antibiotics that also bind to the 50S ribosome, inhibiting peptide bond formation. Chloramphenicol, produced by *Streptomyces venezuelae*, was discovered in 1947; in 1949, it became the first broad-spectrum antibiotic that was approved by the FDA. Although it is a natural antibiotic, it is also easily synthesized and was the first antibacterial drug synthetically mass produced. As a result of its mass production, broad-spectrum coverage, and ability to penetrate into tissues efficiently, chloramphenicol was historically used to treat a wide range of infections, from meningitis to typhoid fever to conjunctivitis. Unfortunately, serious side effects, such as lethal gray baby syndrome, and suppression of bone marrow production, have limited its clinical role. Chloramphenicol also causes anemia in two different ways. One mechanism involves the targeting of mitochondrial ribosomes within hematopoietic stem cells, causing a reversible, dose-dependent suppression of blood cell production. Once chloramphenicol dosing is discontinued, blood cell production returns to normal. This mechanism highlights the similarity between 70S ribosomes of bacteria and the 70S ribosomes within our mitochondria. The second mechanism of anemia is idiosyncratic (i.e., the mechanism is not understood), and involves an irreversible lethal loss of blood cell production known as aplastic anemia. This mechanism of aplastic anemia is not dose dependent and can develop after therapy has stopped. Because of toxicity concerns, chloramphenicol usage in humans is now rare in the United States and is limited to severe infections unable to be treated by less toxic antibiotics. Because its side effects are much less severe in animals, it is used in veterinary medicine.

The **oxazolidinones**, including linezolid, are a new broad-spectrum class of synthetic protein synthesis inhibitors that bind to the 50S ribosomal subunit of both gram-positive and gram-negative bacteria. However, their mechanism of action seems somewhat different from that of the other 50S subunit-binding protein synthesis inhibitors already discussed. Instead, they seem to interfere with formation of the initiation complex (association of the 50S subunit, 30S subunit, and other factors) for translation, and they prevent translocation of the growing protein from the ribosomal A site to the P site. [\[link\]](#) summarizes the protein synthesis inhibitors.

Major classes of protein synthesis-inhibiting antibacterials

Chloramphenicol, macrolides, and lincosamides

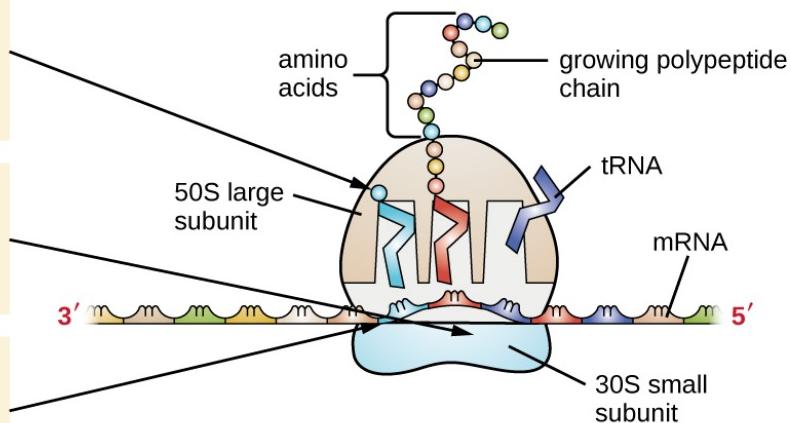
- Bind to the 50S ribosomal subunit
- Prevent peptide bond formation
- Stop protein synthesis

Aminoglycosides

- Bind to the 30S ribosomal subunit
- Impair proofreading, resulting in production of faulty proteins

Tetracyclines

- Bind to the 30S ribosomal subunit
- Block the binding of tRNAs, thereby inhibiting protein synthesis



The major classes of protein synthesis inhibitors target the 30S or 50S subunits of cytoplasmic ribosomes.

Drugs That Inhibit Bacterial Protein Synthesis

Molecular Target	Mechanism of Action	Drug Class	Specific Drugs	Bacteriostatic or Bactericidal	Spectrum of Activity
30S subunit	Causes mismatches between codons and anticodons, leading to faulty proteins that insert into and disrupt cytoplasmic membrane	Aminoglycosides	Streptomycin, gentamicin, neomycin, kanamycin	Bactericidal	Broad spectrum
	Blocks association of tRNAs with ribosome				
		Tetracyclines	Tetracycline, doxycycline, tigecycline	Bacteriostatic	Broad spectrum

Drugs That Inhibit Bacterial Protein Synthesis					
Molecular Target	Mechanism of Action	Drug Class	Specific Drugs	Bacteriostatic or Bactericidal	Spectrum of Activity
	Blocks peptide bond formation between amino acids	Macrolides	Erythromycin, azithromycin, telithromycin	Bacteriostatic	Broad spectrum
		Lincosamides	Lincomycin, clindamycin	Bacteriostatic	Narrow spectrum
		Not applicable	Chloramphenicol	Bacteriostatic	Broad spectrum
50S subunit	Interferes with the formation of the initiation complex between 50S and 30S subunits and other factors.	Oxazolidinones	Linezolid	Bacteriostatic	Broad spectrum

Note:

- Compare and contrast the different types of protein synthesis inhibitors.

Inhibitors of Membrane Function

A small group of antibiotics target the bacterial membrane as their mode of action ([\[link\]](#)). The **polymyxins** are natural polypeptide antibiotics that were first discovered in 1947 as products of *Bacillus polymyxa*; only polymyxin B and polymyxin E (**colistin**) have been used clinically. They are lipophilic with detergent-like properties and interact with the lipopolysaccharide component of the outer membrane of gram-negative bacteria, ultimately disrupting both their outer and inner membranes and killing the bacterial cells. Unfortunately, the membrane-targeting mechanism is not a selective toxicity, and these drugs also target and damage the membrane of cells in the kidney and nervous system when administered systemically. Because of these serious side effects and their poor absorption from the digestive tract, polymyxin B is used in over-the-counter topical antibiotic ointments (e.g., Neosporin), and oral colistin was historically used only for bowel decontamination to prevent infections originating from bowel microbes in immunocompromised patients or for those undergoing certain abdominal surgeries. However, the emergence and spread of multidrug-resistant pathogens has led to increased use of intravenous colistin in hospitals, often as a drug of last resort to treat serious infections. The antibacterial **daptomycin** is a cyclic lipopeptide produced by *Streptomyces roseosporus* that seems to work like the polymyxins, inserting in the bacterial cell membrane and disrupting it. However, in contrast to polymyxin B and colistin, which target only gram-negative bacteria, daptomycin specifically targets gram-positive bacteria. It is

typically administered intravenously and seems to be well tolerated, showing reversible toxicity in skeletal muscles.

Drugs That Inhibit Bacterial Membrane Function

Mechanism of Action	Drug Class	Specific Drugs	Spectrum of Activity	Clinical Use
Interacts with lipopolysaccharide in the outer membrane of gram-negative bacteria, killing the cell through the eventual disruption of the outer membrane and cytoplasmic membrane	Polymyxins	Polymyxin B	Narrow spectrum against gram-negative bacteria, including multidrug-resistant strains	Topical preparations to prevent infections in wounds
		Polymyxin E (colistin)	Narrow spectrum against gram-negative bacteria, including multidrug-resistant strains	Oral dosing to decontaminate bowels to prevent infections in immunocompromised patients or patients undergoing invasive surgery/procedures.
				Intravenous dosing to treat serious systemic infections caused by multidrug-resistant pathogens
Inserts into the cytoplasmic membrane of gram-positive bacteria, disrupting the membrane and killing the cell	Lipopeptide	Daptomycin	Narrow spectrum against gram-positive bacteria, including multidrug-resistant strains	Complicated skin and skin-structure infections and bacteremia caused by gram-positive pathogens, including MRSA

Note:

- How do polymyxins inhibit membrane function?

Inhibitors of Nucleic Acid Synthesis

Some antibacterial drugs work by inhibiting nucleic acid synthesis ([\[link\]](#)). For example, **metronidazole** is a semisynthetic member of the nitroimidazole family that is also an antiprotozoan. It interferes with DNA replication in target cells. The drug **rifampin** is a semisynthetic member of the rifamycin family and functions by blocking RNA polymerase activity in bacteria. The RNA polymerase enzymes in bacteria are structurally different from those in eukaryotes, providing for selective toxicity against bacterial cells. It is used for the treatment of a variety of infections, but its primary use, often in a cocktail with other antibacterial drugs, is against mycobacteria that cause tuberculosis. Despite the selectivity of its mechanism, rifampin can induce liver enzymes to increase metabolism of other drugs being administered (antagonism), leading to hepatotoxicity (liver toxicity) and negatively influencing the bioavailability and therapeutic effect of the companion drugs.

One member of the quinolone family, a group of synthetic antimicrobials, is **nalidixic acid**. It was discovered in 1962 as a byproduct during the synthesis of chloroquine, an antimalarial drug. Nalidixic acid selectively inhibits the activity of bacterial DNA gyrase, blocking DNA replication. Chemical modifications to the original quinolone backbone have resulted in the production of **fluoroquinolones**, like ciprofloxacin and levofloxacin, which also inhibit the activity of DNA gyrase. Ciprofloxacin and levofloxacin are effective against a broad spectrum of gram-positive or gram-negative bacteria, and are among the most commonly prescribed antibiotics used to treat a wide range of infections, including urinary tract infections, respiratory infections, abdominal infections, and skin infections. However, despite their selective toxicity against DNA gyrase, side effects associated with different fluoroquinolones include phototoxicity, neurotoxicity, cardiotoxicity, glucose metabolism dysfunction, and increased risk for tendon rupture.

Drugs That Inhibit Bacterial Nucleic Acid Synthesis				
Mechanisms of Action	Drug Class	Specific Drugs	Spectrum of activity	Clinical Use
Inhibits bacterial RNA polymerase activity and blocks transcription, killing the cell	Rifamycin	Rifampin	Narrow spectrum with activity against gram-positive and limited numbers of gram-negative bacteria. Also active against <i>Mycobacterium tuberculosis</i> .	Combination therapy for treatment of tuberculosis
Inhibits the activity of DNA gyrase and blocks DNA replication, killing the cell	Fluoroquinolones	Ciprofloxacin, ofloxacin, moxifloxacin	Broad spectrum against gram-positive and gram-negative bacteria	Wide variety of skin and systemic infections

Note:

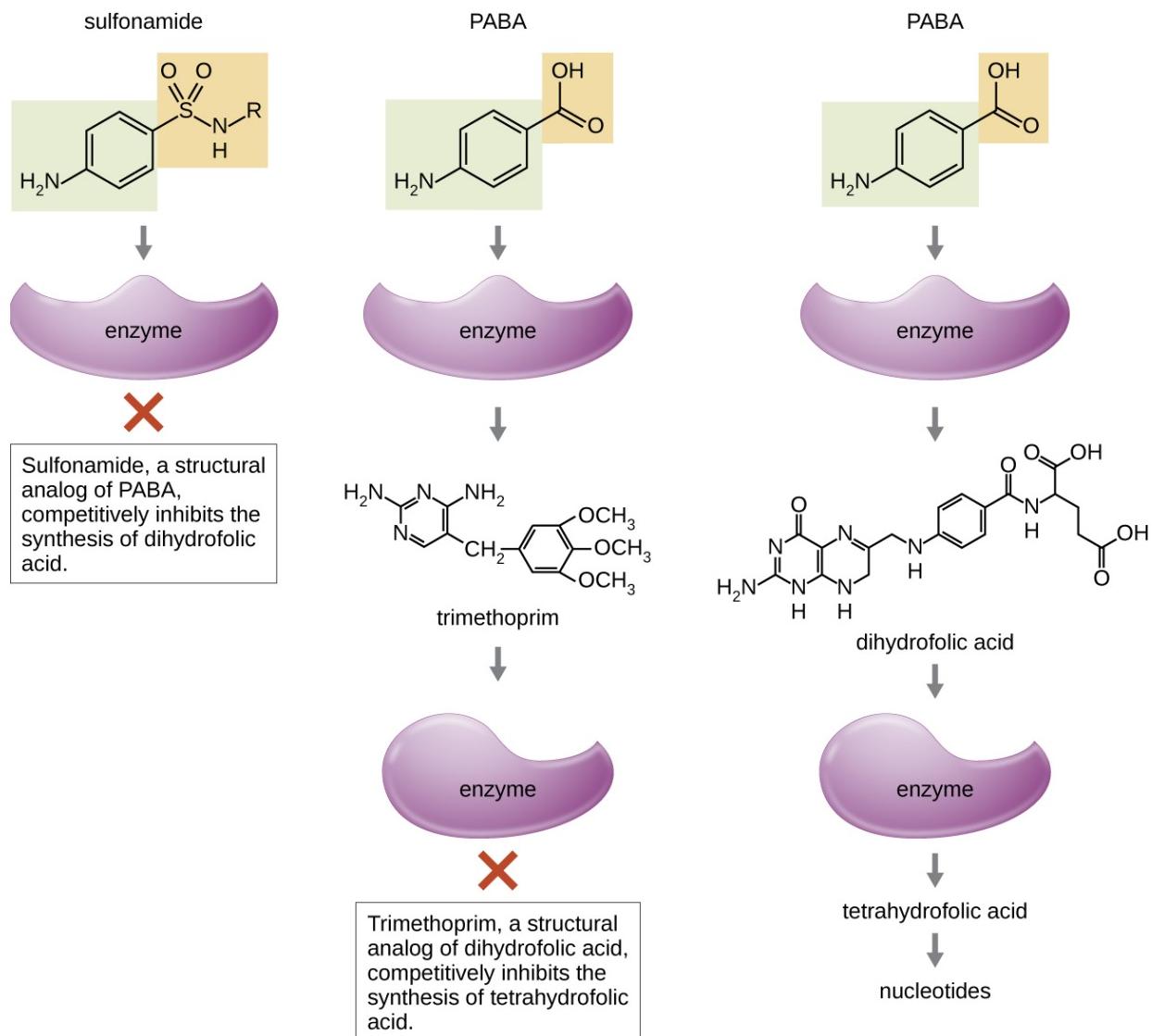
- Why do inhibitors of bacterial nucleic acid synthesis not target host cells?

Inhibitors of Metabolic Pathways

Some synthetic drugs control bacterial infections by functioning as **antimetabolites**, competitive inhibitors for bacterial metabolic enzymes ([\[link\]](#)). The **sulfonamides** (**sulfa drugs**) are the oldest synthetic antibacterial agents and are structural analogues of *para*-aminobenzoic acid (PABA), an early intermediate in folic acid synthesis ([\[link\]](#)). By inhibiting the enzyme involved in the production of dihydrofolic acid, sulfonamides block bacterial biosynthesis of folic acid and, subsequently, pyrimidines and purines required for nucleic acid synthesis. This mechanism of action provides bacteriostatic inhibition of growth against a wide spectrum of gram-positive and gram-negative pathogens. Because humans obtain folic acid from food instead of synthesizing it intracellularly, sulfonamides are selectively toxic for bacteria. However, allergic reactions to sulfa drugs are common. The sulfones are structurally similar to sulfonamides but are not commonly used today except for the treatment of Hansen's disease (leprosy).

Trimethoprim is a synthetic antimicrobial compound that serves as an antimetabolite within the same folic acid synthesis pathway as sulfonamides. However, **trimethoprim** is a structural analogue of dihydrofolic acid and inhibits a later step in the metabolic pathway ([\[link\]](#)). Trimethoprim is used in combination with the sulfa drug sulfamethoxazole to treat urinary tract infections, ear infections, and bronchitis. As discussed, the combination of trimethoprim and sulfamethoxazole is an example of antibacterial synergy. When used alone, each antimetabolite only decreases production of folic acid to a level where bacteriostatic inhibition of growth occurs. However, when used in combination, inhibition of both steps in the metabolic pathway decreases folic acid synthesis to a level that is lethal to the bacterial cell. Because of the importance of folic acid during fetal development, sulfa drugs and trimethoprim use should be carefully considered during early pregnancy.

The drug **isoniazid** is an antimetabolite with specific toxicity for mycobacteria and has long been used in combination with rifampin or streptomycin in the treatment of tuberculosis. It is administered as a prodrug, requiring activation through the action of an intracellular bacterial peroxidase enzyme, forming isoniazid-nicotinamide adenine dinucleotide (NAD) and isoniazid-nicotinamide adenine dinucleotide phosphate (NADP), ultimately preventing the synthesis of mycolic acid, which is essential for mycobacterial cell walls. Possible side effects of isoniazid use include hepatotoxicity, neurotoxicity, and hematologic toxicity (anemia).



Sulfonamides and trimethoprim are examples of antimetabolites that interfere in the bacterial synthesis of folic acid by blocking purine and pyrimidine biosynthesis, thus inhibiting bacterial growth.

Antimetabolite Drugs

Metabolic Pathway Target	Mechanism of Action	Drug Class	Specific Drugs	Spectrum of Activity
Folic acid synthesis	Inhibits the enzyme involved in production of dihydrofolic acid	Sulfonamides	Sulfamethoxazole	Broad spectrum against gram-

Antimetabolite Drugs				
Metabolic Pathway Target	Mechanism of Action	Drug Class	Specific Drugs	Spectrum of Activity
		Sulfones	Dapsone	positive and gram-negative bacteria
	Inhibits the enzyme involved in the production of tetrahydrofolic acid	Not applicable	Trimethoprim	Broad spectrum against gram-positive and gram-negative bacteria
Mycolic acid synthesis	Interferes with the synthesis of mycolic acid	Not applicable	Isoniazid	Narrow spectrum against <i>Mycobacterium</i> spp., including <i>M. tuberculosis</i>

Note:

- How do sulfonamides and trimethoprim selectively target bacteria?

Inhibitor of ATP Synthase

Bedaquiline, representing the synthetic antibacterial class of compounds called the diarylquinolones, uses a novel mode of action that specifically inhibits mycobacterial growth. Although the specific mechanism has yet to be elucidated, this compound appears to interfere with the function of ATP synthases, perhaps by interfering with the use of the hydrogen ion gradient for ATP synthesis by oxidative phosphorylation, leading to reduced ATP production. Due to its side effects, including hepatotoxicity and potentially lethal heart arrhythmia, its use is reserved for serious, otherwise untreatable cases of tuberculosis.

Note:



To learn more about the general principles of antimicrobial therapy and bacterial modes of action, visit [Michigan State University's Antimicrobial Resistance Learning Site](#), particularly pages 6 through 9.

Key Concepts and Summary

- Antibacterial compounds exhibit **selective toxicity**, largely due to differences between prokaryotic and eukaryotic cell structure.
- Cell wall synthesis inhibitors, including the **β -lactams**, the **glycopeptides**, and **bacitracin**, interfere with peptidoglycan synthesis, making bacterial cells more prone to osmotic lysis.
- There are a variety of broad-spectrum, bacterial protein synthesis inhibitors that selectively target the prokaryotic 70S ribosome, including those that bind to the 30S subunit (**aminoglycosides** and **tetracyclines**) and others that bind to the 50S subunit (**macrolides**, **lincosamides**, **chloramphenicol**, and **oxazolidinones**).
- Polymyxins** are lipophilic polypeptide antibiotics that target the lipopolysaccharide component of gram-negative bacteria and ultimately disrupt the integrity of the outer and inner membranes of these bacteria.
- The nucleic acid synthesis inhibitors rifamycins and **fluoroquinolones** target bacterial RNA transcription and DNA replication, respectively.
- Some antibacterial drugs are **antimetabolites**, acting as competitive inhibitors for bacterial metabolic enzymes. **Sulfonamides** and **trimethoprim** are antimetabolites that interfere with bacterial folic acid synthesis. **Isoniazid** is an antimetabolite that interferes with mycolic acid synthesis in mycobacteria.

Short Answer

Exercise:

Problem:

If human cells and bacterial cells perform transcription, how are the rifamycins specific for bacterial infections?

Exercise:

Problem:

What bacterial structural target would make an antibacterial drug selective for gram-negative bacteria? Provide one example of an antimicrobial compound that targets this structure.

Critical Thinking

Exercise:

Problem:

In considering the cell structure of prokaryotes compared with that of eukaryotes, propose one possible reason for side effects in humans due to treatment of bacterial infections with protein synthesis inhibitors.

Mechanisms of Other Antimicrobial Drugs

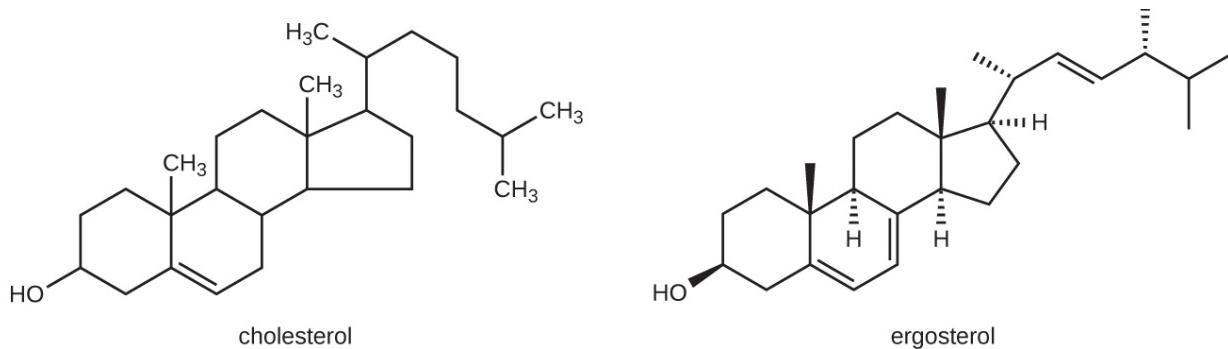
LEARNING OBJECTIVE

- Explain the differences between modes of action of drugs that target fungi, protozoa, helminths, and viruses

Because fungi, protozoa, and helminths are eukaryotic, their cells are very similar to human cells, making it more difficult to develop drugs with selective toxicity. Additionally, viruses replicate within human host cells, making it difficult to develop drugs that are selectively toxic to viruses or virus-infected cells. Despite these challenges, there are antimicrobial drugs that target fungi, protozoa, helminths, and viruses, and some even target more than one type of microbe. [\[link\]](#), [\[link\]](#), [\[link\]](#), and [\[link\]](#) provide examples for antimicrobial drugs in these various classes.

Antifungal Drugs

The most common mode of action for antifungal drugs is the disruption of the cell membrane. Antifungals take advantage of small differences between fungi and humans in the biochemical pathways that synthesize sterols. The sterols are important in maintaining proper membrane fluidity and, hence, proper function of the cell membrane. For most fungi, the predominant membrane sterol is ergosterol. Because human cell membranes use cholesterol, instead of ergosterol, antifungal drugs that target ergosterol synthesis are selectively toxic ([\[link\]](#)).



The predominant sterol found in human cells is cholesterol, whereas the predominant sterol found in fungi is ergosterol, making ergosterol a good target for antifungal drug development.

The **imidazoles** are synthetic fungicides that disrupt ergosterol biosynthesis; they are commonly used in medical applications and also in agriculture to keep seeds and harvested crops from molding. Examples include miconazole, ketoconazole, and clotrimazole, which are used to treat fungal skin infections such as ringworm, specifically tinea pedis (athlete's foot), tinea cruris (jock itch), and tinea corporis. These infections are commonly caused by dermatophytes of the genera *Trichophyton*, *Epidermophyton*, and *Microsporum*. Miconazole is also used predominantly for the treatment of vaginal yeast infections caused by the fungus *Candida*, and ketoconazole is used for the treatment of tinea versicolor and dandruff, which both can be caused by the fungus *Malassezia*.

The **triazole** drugs, including **fluconazole**, also inhibit ergosterol biosynthesis. However, they can be administered orally or intravenously for the treatment of several types of systemic yeast infections, including oral thrush and cryptococcal meningitis, both of which are prevalent in patients with AIDS. The triazoles also exhibit more selective toxicity, compared with the imidazoles, and are associated with fewer side effects.

The **allylamines**, a structurally different class of synthetic antifungal drugs, inhibit an earlier step in ergosterol biosynthesis. The most commonly used allylamine is **terbinafine** (marketed under the brand name Lamisil), which is used topically for the treatment of dermatophytic skin infections like athlete's foot, ringworm, and jock itch. Oral treatment with terbinafine is also used for the treatment of fingernail and toenail fungus, but it can be associated with the rare side effect of hepatotoxicity.

The **polyenes** are a class of antifungal agents naturally produced by certain actinomycete soil bacteria and are structurally related to macrolides. These large,

lipophilic molecules bind to ergosterol in fungal cytoplasmic membranes, thus creating pores. Common examples include nystatin and amphotericin B. Nystatin is typically used as a topical treatment for yeast infections of the skin, mouth, and vagina, but may also be used for intestinal fungal infections. The drug **amphotericin B** is used for systemic fungal infections like aspergillosis, cryptococcal meningitis, histoplasmosis, blastomycosis, and candidiasis. Amphotericin B was the only antifungal drug available for several decades, but its use is associated with some serious side effects, including nephrotoxicity (kidney toxicity).

Amphotericin B is often used in combination with flucytosine, a fluorinated pyrimidine analog that is converted by a fungal-specific enzyme into a toxic product that interferes with both DNA replication and protein synthesis in fungi. Flucytosine is also associated with hepatotoxicity (liver toxicity) and bone marrow depression.

Beyond targeting ergosterol in fungal cell membranes, there are a few antifungal drugs that target other fungal structures ([\[link\]](#)). The echinocandins, including caspofungin, are a group of naturally produced antifungal compounds that block the synthesis of $\beta(1 \rightarrow 3)$ glucan found in fungal cell walls but not found in human cells. This drug class has the nickname “penicillin for fungi.” Caspofungin is used for the treatment of aspergillosis as well as systemic yeast infections.

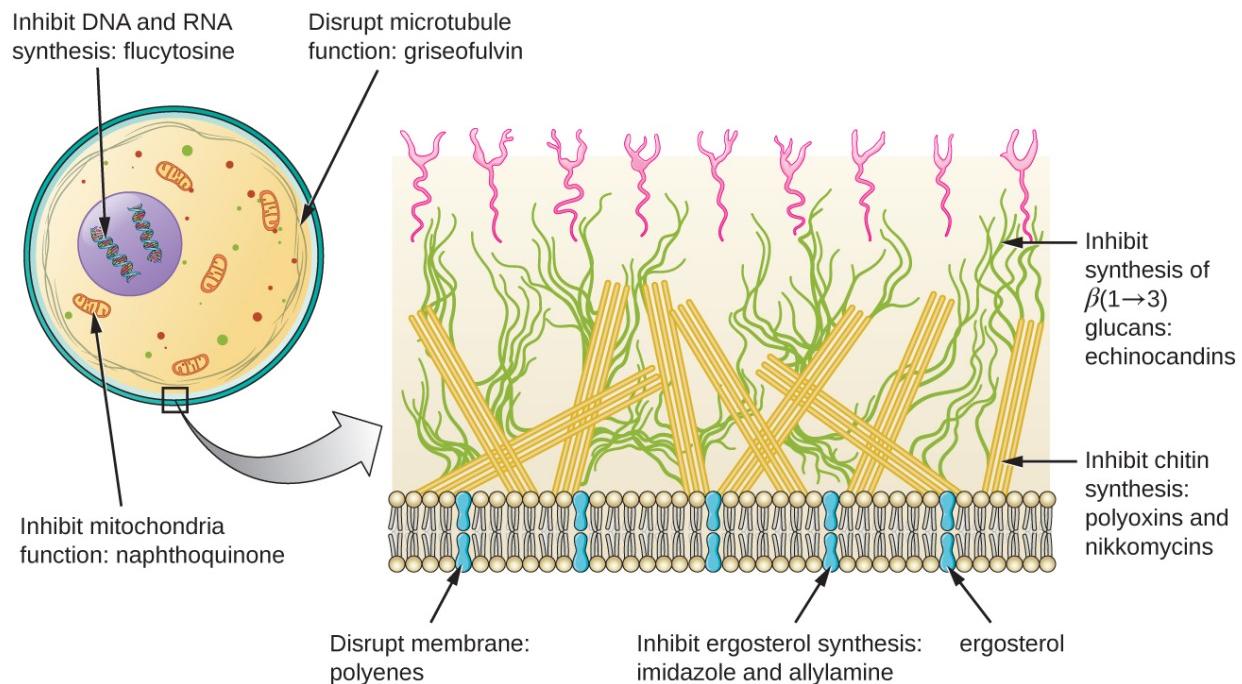
Although chitin is only a minor constituent of fungal cell walls, it is also absent in human cells, making it a selective target. The polyoxins and nikkomycins are naturally produced antifungals that target chitin synthesis. Polyoxins are used to control fungi for agricultural purposes, and nikkomycin Z is currently under development for use in humans to treat yeast infections and Valley fever (coccidioidomycosis), a fungal disease prevalent in the southwestern US. [\[footnote\]](#) Centers for Disease Control and Prevention. “Valley Fever: Awareness Is Key.” <http://www.cdc.gov/features/valleyfever/>. Accessed June 1, 2016.

The naturally produced antifungal griseofulvin is thought to specifically disrupt fungal cell division by interfering with microtubules involved in spindle formation during mitosis. It was one of the first antifungals, but its use is associated with hepatotoxicity. It is typically administered orally to treat various types of dermatophytic skin infections when other topical antifungal treatments are ineffective.

There are a few drugs that act as antimetabolites against fungal processes. For example, atovaquone, a representative of the naphthoquinone drug class, is a semisynthetic antimetabolite for fungal and protozoal versions of a mitochondrial cytochrome important in electron transport. Structurally, it is an analog of coenzyme Q, with which it competes for electron binding. It is particularly useful for the

treatment of *Pneumocystis* pneumonia caused by *Pneumocystis jirovecii*. The antibacterial sulfamethoxazole-trimethoprim combination also acts as an antimetabolite against *P. jirovecii*.

[\[link\]](#) shows the various therapeutic classes of antifungal drugs, categorized by mode of action, with examples of each.



Antifungal drugs target several different cell structures. (credit right: modification of work by “Maya and Rike”/Wikimedia Commons)

Common Antifungal Drugs

Mechanism of Action	Drug Class	Specific Drugs	Clinical Uses

Common Antifungal Drugs			
Mechanism of Action	Drug Class	Specific Drugs	Clinical Uses
Inhibit ergosterol synthesis	Imidazoles	Miconazole, ketoconazole, clotrimazole	Fungal skin infections and vaginal yeast infections
	Triazoles	Fluconazole	Systemic yeast infections, oral thrush, and cryptococcal meningitis
	Allylamines	Terbinafine	Dermatophytic skin infections (athlete's foot, ring worm, jock itch), and infections of fingernails and toenails
Bind ergosterol in the cell membrane and create pores that disrupt the membrane	Polyenes	Nystatin	Used topically for yeast infections of skin, mouth, and vagina; also used for fungal infections of the intestine
		Amphotericin B	Variety systemic fungal infections
Inhibit cell wall synthesis	Echinocandins	Caspofungin	Aspergillosis and systemic yeast infections
	Not applicable	Nikkomycin Z	Coccidioidomycosis (Valley fever) and yeast infections

Common Antifungal Drugs

Mechanism of Action	Drug Class	Specific Drugs	Clinical Uses
Inhibit microtubules and cell division	Not applicable	Griseofulvin	Dermatophytic skin infections

Note:

- How is disruption of ergosterol biosynthesis an effective mode of action for antifungals?

Note:

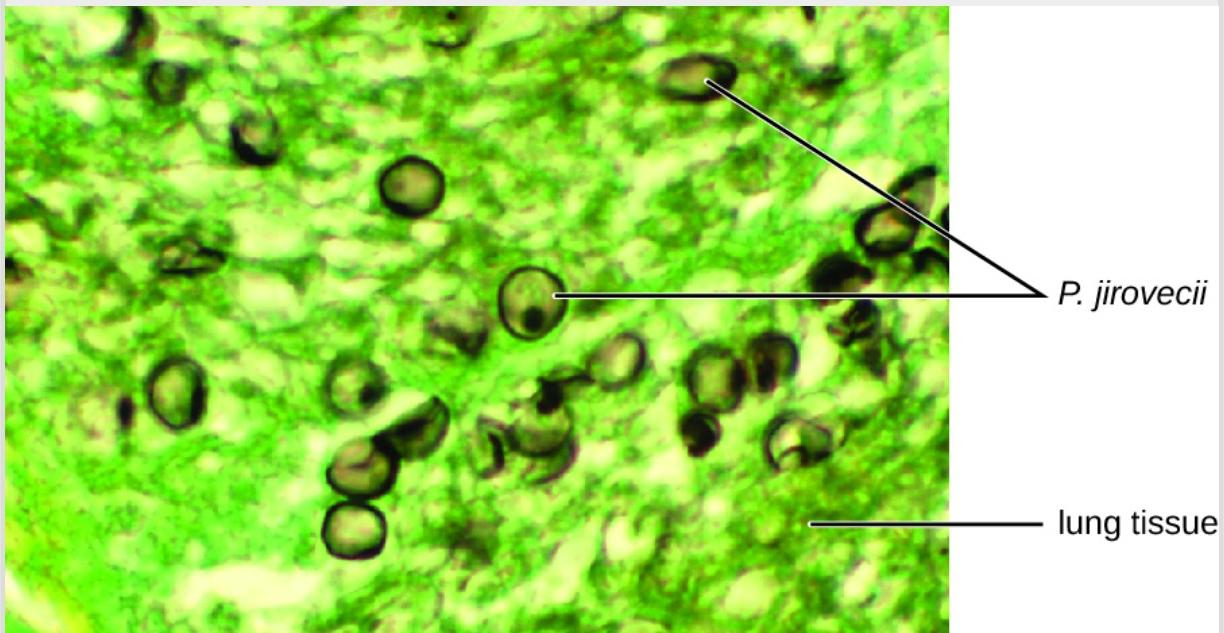
Treating a Fungal Infection of the Lungs

Jack, a 48-year-old engineer, is HIV positive but generally healthy thanks to antiretroviral therapy (ART). However, after a particularly intense week at work, he developed a fever and a dry cough. He assumed that he just had a cold or mild flu due to overexertion and didn't think much of it. However, after about a week, he began to experience fatigue, weight loss, and shortness of breath. He decided to visit his physician, who found that Jack had a low level of blood oxygenation. The physician ordered blood testing, a chest X-ray, and the collection of an induced sputum sample for analysis. His X-ray showed a fine cloudiness and several pneumatoceles (thin-walled pockets of air), which indicated *Pneumocystis* pneumonia (PCP), a type of pneumonia caused by the fungus *Pneumocystis jirovecii*. Jack's physician admitted him to the hospital and prescribed Bactrim, a combination of sulfamethoxazole and trimethoprim, to be administered intravenously.

P. jirovecii is a yeast-like fungus with a life cycle similar to that of protozoans. As such, it was classified as a protozoan until the 1980s. It lives only in the lung tissue of infected persons and is transmitted from person to person, with many people exposed as children. Typically, *P. jirovecii* only causes pneumonia in immunocompromised individuals. Healthy people may carry the fungus in their

lungs with no symptoms of disease. PCP is particularly problematic among HIV patients with compromised immune systems.

PCP is usually treated with oral or intravenous Bactrim, but atovaquone or pentamidine (another antiparasitic drug) are alternatives. If not treated, PCP can progress, leading to a collapsed lung and nearly 100% mortality. Even with antimicrobial drug therapy, PCP still is responsible for 10% of HIV-related deaths. The cytological examination, using direct immunofluorescence assay (DFA), of a smear from Jack's sputum sample confirmed the presence of *P. jirovecii* ([\[link\]](#)). Additionally, the results of Jack's blood tests revealed that his white blood cell count had dipped, making him more susceptible to the fungus. His physician reviewed his ART regimen and made adjustments. After a few days of hospitalization, Jack was released to continue his antimicrobial therapy at home. With the adjustments to his ART therapy, Jack's CD4 counts began to increase and he was able to go back to work.



Microscopic examination of an induced sputum sample or bronchoaveolar lavage sample typically reveals the organism, as shown here. (credit: modification of work by the Centers for Disease Control and Prevention)

Antiprotozoan Drugs

There are a few mechanisms by which antiprotozoan drugs target infectious protozoans ([\[link\]](#)). Some are antimetabolites, such as atovaquone, proguanil, and artemisinins. Atovaquone, in addition to being antifungal, blocks electron transport in protozoans and is used for the treatment of protozoan infections including malaria, babesiosis, and toxoplasmosis. Proguanil is another synthetic antimetabolite that is processed in parasitic cells into its active form, which inhibits protozoan folic acid synthesis. It is often used in combination with atovaquone, and the combination is marketed as Malarone for both malaria treatment and prevention.

Artemisinin, a plant-derived antifungal first discovered by Chinese scientists in the 1970s, is quite effective against malaria. Semisynthetic derivatives of **artemisinin** are more water soluble than the natural version, which makes them more bioavailable. Although the exact mechanism of action is unclear, artemisinins appear to act as prodrugs that are metabolized by target cells to produce reactive oxygen species (ROS) that damage target cells. Due to the rise in resistance to antimalarial drugs, artemisinins are also commonly used in combination with other antimalarial compounds in artemisinin-based combination therapy (ACT).

Several antimetabolites are used for the treatment of toxoplasmosis caused by the parasite *Toxoplasma gondii*. The synthetic sulfa drug sulfadiazine competitively inhibits an enzyme in folic acid production in parasites and can be used to treat malaria and toxoplasmosis. Pyrimethamine is a synthetic drug that inhibits a different enzyme in the folic acid production pathway and is often used in combination with sulfadoxine (another sulfa drug) for the treatment of malaria or in combination with sulfadiazine for the treatment of toxoplasmosis. Side effects of pyrimethamine include decreased bone marrow activity that may cause increased bruising and low red blood cell counts. When toxicity is a concern, spiramycin, a macrolide protein synthesis inhibitor, is typically administered for the treatment of toxoplasmosis.

Two classes of antiprotozoan drugs interfere with nucleic acid synthesis: nitroimidazoles and quinolines. Nitroimidazoles, including semisynthetic metronidazole, which was discussed previously as an antibacterial drug, and synthetic tinidazole, are useful in combating a wide variety of protozoan pathogens, such as *Giardia lamblia*, *Entamoeba histolytica*, and *Trichomonas vaginalis*. Upon introduction into these cells in low-oxygen environments, nitroimidazoles become activated and introduce DNA strand breakage, interfering with DNA replication in target cells. Unfortunately, metronidazole is associated with carcinogenesis (the development of cancer) in humans.

Another type of synthetic antiprotozoan drug that has long been thought to specifically interfere with DNA replication in certain pathogens is **pentamidine**. It has historically been used for the treatment of African sleeping sickness (caused by

the protozoan *Trypanosoma brucei*) and leishmaniasis (caused by protozoa of the genus *Leishmania*), but it is also an alternative treatment for the fungus *Pneumocystis*. Some studies indicate that it specifically binds to the DNA found within kinetoplasts (kDNA; long mitochondrion-like structures unique to trypanosomes), leading to the cleavage of kDNA. However, nuclear DNA of both the parasite and host remain unaffected. It also appears to bind to tRNA, inhibiting the addition of amino acids to tRNA, thus preventing protein synthesis. Possible side effects of pentamidine use include pancreatic dysfunction and liver damage.

The **quinolines** are a class of synthetic compounds related to quinine, which has a long history of use against malaria. Quinolines are thought to interfere with heme detoxification, which is necessary for the parasite's effective breakdown of hemoglobin into amino acids inside red blood cells. The synthetic derivatives chloroquine, quinacrine (also called mepacrine), and mefloquine are commonly used as antimalarials, and chloroquine is also used to treat amoebiasis typically caused by *Entamoeba histolytica*. Long-term prophylactic use of chloroquine or mefloquine may result in serious side effects, including hallucinations or cardiac issues. Patients with glucose-6-phosphate dehydrogenase deficiency experience severe anemia when treated with chloroquine.

Common Antiprotozoan Drugs

Mechanism of Action	Drug Class	Specific Drugs	Clinical Uses
Inhibit electron transport in mitochondria	Naphthoquinone	Atovaquone	Malaria, babesiosis, and toxoplasmosis
Inhibit folic acid synthesis	Not applicable	Proquanil	Combination therapy with atovaquone for malaria treatment and prevention

Common Antiprotozoan Drugs			
Mechanism of Action	Drug Class	Specific Drugs	Clinical Uses
Inhibits dihydrofolate reductase	Sulfonamide	Sulfadiazine	Malaria and toxoplasmosis
	Not applicable	Pyrimethamine	Combination therapy with sulfadoxine (sulfa drug) for malaria
Produces damaging reactive oxygen species	Not applicable	Artemisinin	Combination therapy to treat malaria
Inhibit DNA synthesis	Nitroimidazoles	Metronidazole, tinidazole	Infections caused by <i>Giardia lamblia</i> , <i>Entamoeba histolytica</i> , and <i>Trichomonas vaginalis</i>
	Not applicable	Pentamidine	African sleeping sickness and leishmaniasis
Inhibit heme detoxification	Quinolines	Chloroquine	Malaria and infections with <i>E. histolytica</i>
		Mepacrine, mefloquine	Malaria

Note:

- List two modes of action for antiprotozoan drugs.

Antihelminthic Drugs

Because helminths are multicellular eukaryotes like humans, developing drugs with selective toxicity against them is extremely challenging. Despite this, several effective classes have been developed ([\[link\]](#)). Synthetic **benzimidazoles**, like **mebendazole** and **albendazole**, bind to helminthic β -tubulin, preventing microtubule formation. Microtubules in the intestinal cells of the worms seem to be particularly affected, leading to a reduction in glucose uptake. Besides their activity against a broad range of helminths, benzimidazoles are also active against many protozoans, fungi, and viruses, and their use for inhibiting mitosis and cell cycle progression in cancer cells is under study.[\[footnote\]](#) Possible side effects of their use include liver damage and bone marrow suppression.

B. Chu et al. “A Benzimidazole Derivative Exhibiting Antitumor Activity Blocks EGFR and HER2 Activity and Upregulates DR5 in Breast Cancer Cells.” *Cell Death and Disease* 6 (2015):e1686

The avermectins are members of the macrolide family that were first discovered from a Japanese soil isolate, *Streptomyces avermectinii*. A more potent semisynthetic derivative of avermectin is **ivermectin**, which binds to glutamate-gated chloride channels specific to invertebrates including helminths, blocking neuronal transmission and causing starvation, paralysis, and death of the worms. Ivermectin is used to treat roundworm diseases, including onchocerciasis (also called river blindness, caused by the worm *Onchocerca volvulus*) and strongyloidiasis (caused by the worm *Strongyloides stercoralis* or *S. fuelleborni*). Ivermectin also can also treat parasitic insects like mites, lice, and bed bugs, and is nontoxic to humans.

Niclosamide is a synthetic drug that has been used for over 50 years to treat tapeworm infections. Although its mode of action is not entirely clear, niclosamide appears to inhibit ATP formation under anaerobic conditions and inhibit oxidative phosphorylation in the mitochondria of its target pathogens. Niclosamide is not absorbed from the gastrointestinal tract, thus it can achieve high localized intestinal concentrations in patients. Recently, it has been shown to also have antibacterial, antiviral, and antitumor activities.[\[footnote\]](#)[\[footnote\]](#)[\[footnote\]](#)

J.-X. Pan et al. “Niclosamide, An Old Antihelminthic Agent, Demonstrates Antitumor Activity by Blocking Multiple Signaling Pathways of Cancer Stem Cells.”

Chinese Journal of Cancer 31 no. 4 (2012):178–184.

F. Imperi et al. “New Life for an Old Drug: The Anthelmintic Drug Niclosamide Inhibits *Pseudomonas aeruginosa* Quorum Sensing.” *Antimicrobial Agents and Chemotherapy* 57 no. 2 (2013):996-1005.

A. Jurgeit et al. “Niclosamide Is a Proton Carrier and Targets Acidic Endosomes with Broad Antiviral Effects.” *PLoS Pathogens* 8 no. 10 (2012):e1002976.

Another synthetic antihelminthic drug is **praziquantel**, which used for the treatment of parasitic tapeworms and liver flukes, and is particularly useful for the treatment of schistosomiasis (caused by blood flukes from three genera of *Schistosoma*). Its mode of action remains unclear, but it appears to cause the influx of calcium into the worm, resulting in intense spasm and paralysis of the worm. It is often used as a preferred alternative to niclosamide in the treatment of tapeworms when gastrointestinal discomfort limits niclosamide use.

The thioxanthenones, another class of synthetic drugs structurally related to quinine, exhibit antischistosomal activity by inhibiting RNA synthesis. The thioxanthone lucanthone and its metabolite hycanthone were the first used clinically, but serious neurological, gastrointestinal, cardiovascular, and hepatic side effects led to their discontinuation. Oxamniquine, a less toxic derivative of hycanthone, is only effective against *S. mansoni*, one of the three species known to cause schistosomiasis in humans. Praziquantel was developed to target the other two schistosome species, but concerns about increasing resistance have renewed interest in developing additional derivatives of oxamniquine to target all three clinically important schistosome species.

Common Antihelminthic Drugs

Mechanism of Action	Drug Class	Specific Drugs	Clinical Uses
Inhibit microtubule formation, reducing glucose uptake	Benzimidazoles	Mebendazole, albendazole	Variety of helminth infections

Common Antihelminthic Drugs			
Mechanism of Action	Drug Class	Specific Drugs	Clinical Uses
Block neuronal transmission, causing paralysis and starvation	Avermectins	Ivermectin	Roundworm diseases, including river blindness and strongyloidiasis, and treatment of parasitic insects
Inhibit ATP production	Not applicable	Niclosamide	Intestinal tapeworm infections
Induce calcium influx	Not applicable	Praziquantel	Schistosomiasis (blood flukes)
Inhibit RNA synthesis	Thioxanthenones	Lucanthone, hycanthone, oxamniquine	Schistosomiasis (blood flukes)

Note:

- Why are antihelminthic drugs difficult to develop?

Antiviral Drugs

Unlike the complex structure of fungi, protozoa, and helminths, viral structure is simple, consisting of nucleic acid, a protein coat, viral enzymes, and, sometimes, a lipid envelope. Furthermore, viruses are obligate intracellular pathogens that use the host's cellular machinery to replicate. These characteristics make it difficult to develop drugs with selective toxicity against viruses.

Many antiviral drugs are nucleoside analogs and function by inhibiting nucleic acid biosynthesis. For example, **acyclovir** (marketed as Zovirax) is a synthetic analog of the nucleoside guanosine ([\[link\]](#)). It is activated by the herpes simplex viral enzyme thymidine kinase and, when added to a growing DNA strand during replication, causes chain termination. Its specificity for virus-infected cells comes from both the need for a viral enzyme to activate it and the increased affinity of the activated form for viral DNA polymerase compared to host cell DNA polymerase. Acyclovir and its derivatives are frequently used for the treatment of herpes virus infections, including genital herpes, chickenpox, shingles, Epstein-Barr virus infections, and cytomegalovirus infections. Acyclovir can be administered either topically or systemically, depending on the infection. One possible side effect of its use includes nephrotoxicity. The drug adenine-arabinoside, marketed as vidarabine, is a synthetic analog to deoxyadenosine that has a mechanism of action similar to that of acyclovir. It is also effective for the treatment of various human herpes viruses. However, because of possible side effects involving low white blood cell counts and neurotoxicity, treatment with acyclovir is now preferred.

Ribavirin, another synthetic guanosine analog, works by a mechanism of action that is not entirely clear. It appears to interfere with both DNA and RNA synthesis, perhaps by reducing intracellular pools of guanosine triphosphate (GTP). Ribavirin also appears to inhibit the RNA polymerase of hepatitis C virus. It is primarily used for the treatment of the RNA viruses like hepatitis C (in combination therapy with interferon) and respiratory syncytial virus. Possible side effects of ribavirin use include anemia and developmental effects on unborn children in pregnant patients. In recent years, another nucleotide analog, sofosbuvir (Solvaldi), has also been developed for the treatment of hepatitis C. Sofosbuvir is a uridine analog that interferes with viral polymerase activity. It is commonly coadministered with ribavirin, with and without interferon.

Inhibition of nucleic acid synthesis is not the only target of synthetic antivirals. Although the mode of action of **amantadine** and its relative **rimantadine** are not entirely clear, these drugs appear to bind to a transmembrane protein that is involved in the escape of the influenza virus from endosomes. Blocking escape of the virus also prevents viral RNA release into host cells and subsequent viral replication. Increasing resistance has limited the use of amantadine and rimantadine in the treatment of influenza A. Use of amantadine can result in neurological side effects, but the side effects of rimantadine seem less severe. Interestingly, because of their effects on brain chemicals such as dopamine and NMDA (N-methyl D-aspartate), amantadine and rimantadine are also used for the treatment of Parkinson's disease.

Neuraminidase inhibitors, including oseltamivir (Tamiflu), zanamivir (Relenza), and peramivir (Rapivab), specifically target influenza viruses by blocking the activity of

influenza virus neuraminidase, preventing the release of the virus from infected cells. These three antivirals can decrease flu symptoms and shorten the duration of illness, but they differ in their modes of administration: oseltamivir is administered orally, zanamivir is inhaled, and peramivir is administered intravenously. Resistance to these neuraminidase inhibitors still seems to be minimal.

Pleconaril is a synthetic antiviral under development that showed promise for the treatment of picornaviruses. Use of **pleconaril** for the treatment of the common cold caused by rhinoviruses was not approved by the FDA in 2002 because of lack of proven effectiveness, lack of stability, and association with irregular menstruation. Its further development for this purpose was halted in 2007. However, pleconaril is still being investigated for use in the treatment of life-threatening complications of enteroviruses, such as meningitis and sepsis. It is also being investigated for use in the global eradication of a specific enterovirus, polio.[\[footnote\]](#) Pleconaril seems to work by binding to the viral capsid and preventing the uncoating of viral particles inside host cells during viral infection.

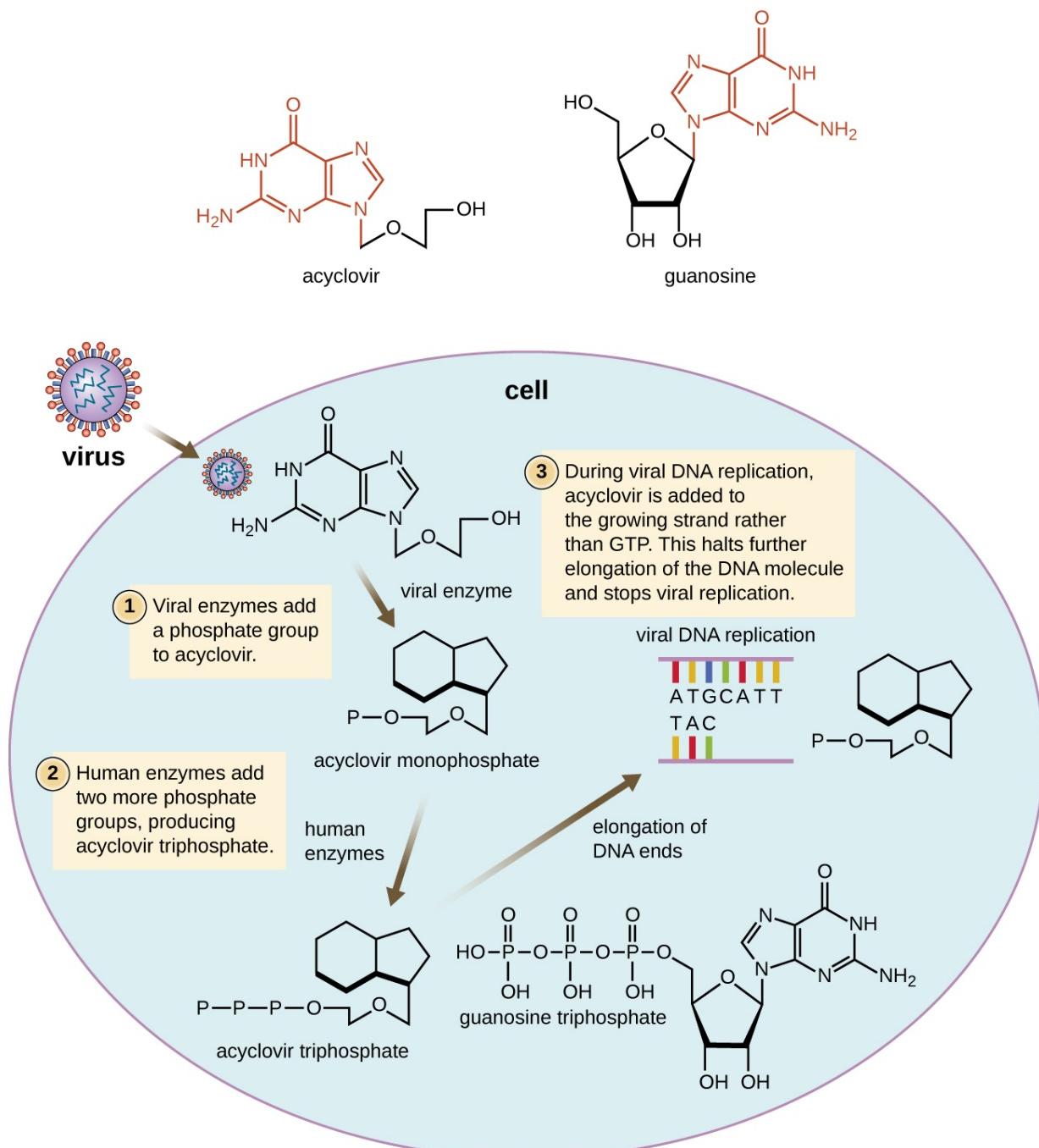
M.J. Abzug. “The Enteroviruses: Problems in Need of Treatments.” *Journal of Infection* 68 no. S1 (2014):108–14.

Viruses with complex life cycles, such as HIV, can be more difficult to treat. First, HIV targets CD4-positive white blood cells, which are necessary for a normal immune response to infection. Second, HIV is a retrovirus, meaning that it converts its RNA genome into a DNA copy that integrates into the host cell’s genome, thus hiding within host cell DNA. Third, the HIV reverse transcriptase lacks proofreading activity and introduces mutations that allow for rapid development of antiviral drug resistance. To help prevent the emergence of resistance, a combination of specific synthetic antiviral drugs is typically used in ART for HIV ([\[link\]](#)).

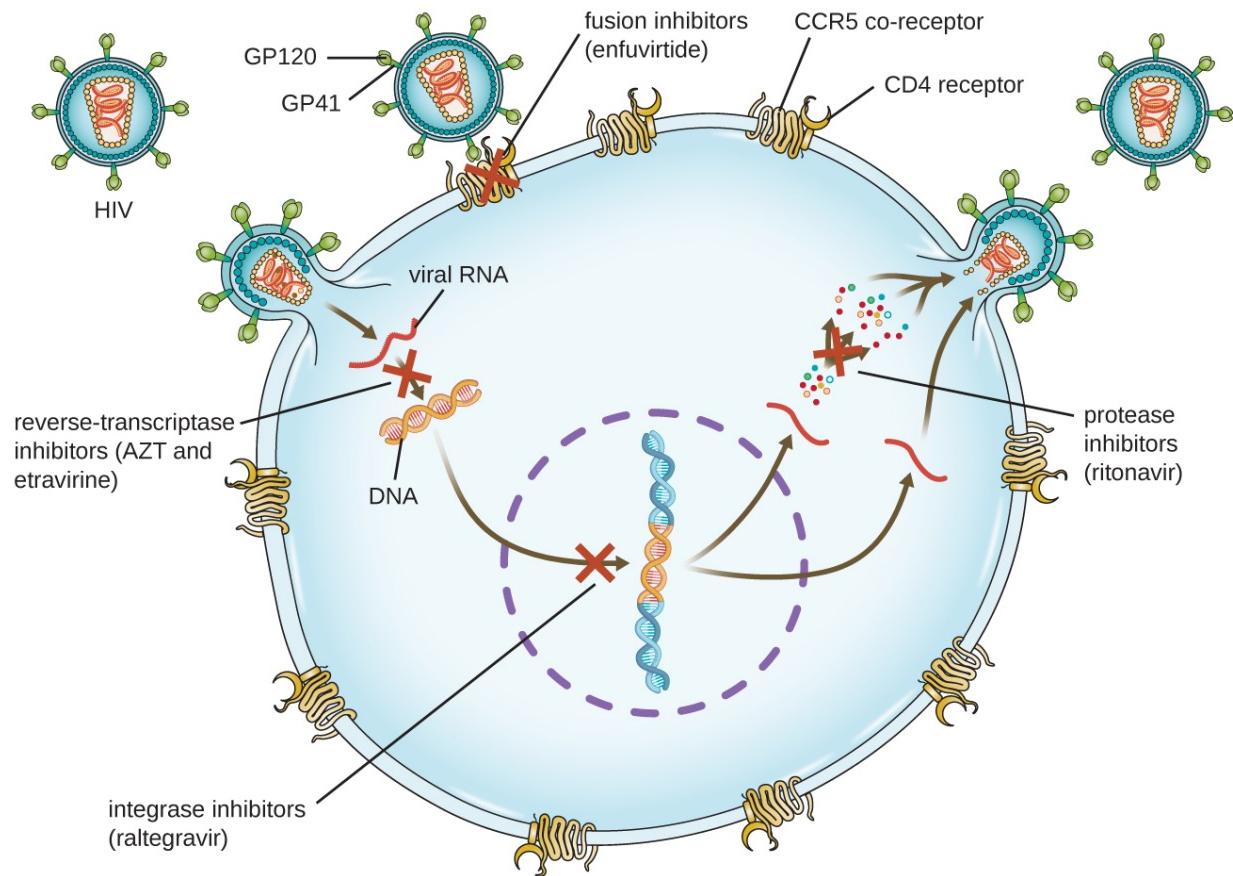
The **reverse transcriptase inhibitors** block the early step of converting viral RNA genome into DNA, and can include competitive nucleoside analog inhibitors (e.g., azidothymidine/zidovudine, or AZT) and non-nucleoside noncompetitive inhibitors (e.g., etravirine) that bind reverse transcriptase and cause an inactivating conformational change. Drugs called **protease inhibitors** (e.g., ritonavir) block the processing of viral proteins and prevent viral maturation. Protease inhibitors are also being developed for the treatment of other viral types.[\[footnote\]](#) For example, simeprevir (Olysio) has been approved for the treatment of hepatitis C and is administered with ribavirin and interferon in combination therapy. The **integrase inhibitors** (e.g., raltegravir), block the activity of the HIV integrase responsible for the recombination of a DNA copy of the viral genome into the host cell chromosome. Additional drug classes for HIV treatment include the CCR5 antagonists and the **fusion inhibitors** (e.g., enfuvirtide), which prevent the binding of HIV to the host cell coreceptor (chemokine receptor type 5 [CCR5]) and the merging of the viral

envelope with the host cell membrane, respectively. [link] shows the various therapeutic classes of antiviral drugs, categorized by mode of action, with examples of each.

B.L. Pearlman. "Protease Inhibitors for the Treatment of Chronic Hepatitis C Genotype-1 Infection: The New Standard of Care." *Lancet Infectious Diseases* 12 no. 9 (2012):717–728.



Acyclovir is a structural analog of guanosine. It is specifically activated by the viral enzyme thymidine kinase and then preferentially binds to viral DNA polymerase, leading to chain termination during DNA replication.



Antiretroviral therapy (ART) is typically used for the treatment of HIV. The targets of drug classes currently in use are shown here. (credit: modification of work by Thomas Splettstoesser)

Common Antiviral Drugs		
Mechanism of Action	Drug	Clinical Uses
Nucleoside analog inhibition of nucleic acid synthesis	Acyclovir	Herpes virus infections
	Azidothymidine/zidovudine (AZT)	HIV infections
	Ribavirin	Hepatitis C virus and respiratory syncytial virus infections
	Vidarabine	Herpes virus infections
	Sofosbuvir	Hepatitis C virus infections
Non-nucleoside noncompetitive inhibition	Etravirine	HIV infections
Inhibit escape of virus from endosomes	Amantadine, rimantadine	Infections with influenza virus
Inhibit neuraminidase	Olsetamivir, zanamivir, peramivir	Infections with influenza virus
Inhibit viral uncoating	Pleconaril	Serious enterovirus infections
Inhibition of protease	Ritonavir	HIV infections
	Simeprevir	Hepatitis C virus infections

Common Antiviral Drugs

Mechanism of Action	Drug	Clinical Uses
Inhibition of integrase	Raltegravir	HIV infections
Inhibition of membrane fusion	Enfuvirtide	HIV infections

Note:

- Why is HIV difficult to treat with antivirals?

Note:



To learn more about the various classes of antiretroviral drugs used in the ART of HIV infection, explore each of the drugs in the HIV drug classes provided by US Department of Health and Human Services at [this](#) website.

Key Concepts and Summary

- Because fungi, protozoans, and helminths are eukaryotic organisms like human cells, it is more challenging to develop antimicrobial drugs that specifically target them. Similarly, it is hard to target viruses because human viruses replicate inside of human cells.
- **Antifungal drugs** interfere with ergosterol synthesis, bind to ergosterol to disrupt fungal cell membrane integrity, or target cell wall-specific components

or other cellular proteins.

- **Antiprotozoan drugs** increase cellular levels of reactive oxygen species, interfere with protozoal DNA replication (nuclear versus kDNA, respectively), and disrupt heme detoxification.
- **Antihelminthic drugs** disrupt helminthic and protozoan microtubule formation; block neuronal transmissions; inhibit anaerobic ATP formation and/or oxidative phosphorylation; induce a calcium influx in tapeworms, leading to spasms and paralysis; and interfere with RNA synthesis in schistosomes.
- **Antiviral drugs** inhibit viral entry, inhibit viral uncoating, inhibit nucleic acid biosynthesis, prevent viral escape from endosomes in host cells, and prevent viral release from infected cells.
- Because it can easily mutate to become drug resistant, HIV is typically treated with a combination of several **antiretroviral drugs**, which may include **reverse transcriptase inhibitors, protease inhibitors, integrase inhibitors**, and drugs that interfere with viral binding and fusion to initiate infection.

Multiple Choice

Exercise:

Problem:

Which of the following is not an appropriate target for antifungal drugs?

- A. ergosterol
- B. chitin
- C. cholesterol
- D. $\beta(1 \rightarrow 3)$ glucan

Solution:

C

Exercise:

Problem:

Which of the following drug classes specifically inhibits neuronal transmission in helminths?

- A. quinolines
- B. avermectins
- C. amantadines

D. imidazoles

Solution:

B

Exercise:

Problem:

Which of the following is a nucleoside analog commonly used as a reverse transcriptase inhibitor in the treatment of HIV?

- A. acyclovir
 - B. ribavirin
 - C. adenine-arabinoside
 - D. azidothymidine
-

Solution:

D

Exercise:

Problem:

Which of the following is an antimalarial drug that is thought to increase ROS levels in target cells?

- A. artemisinin
 - B. amphotericin b
 - C. praziquantel
 - D. pleconaril
-

Solution:

A

Fill in the Blank

Exercise:

Problem:

Antiviral drugs, like Tamiflu and Relenza, that are effective against the influenza virus by preventing viral escape from host cells are called _____.

Solution:

neuraminidase inhibitors

True/False**Exercise:****Problem:**

Echinocandins, known as “penicillin for fungi,” target $\beta(1 \rightarrow 3)$ glucan in fungal cell walls.

Solution:

true

Short Answer**Exercise:****Problem:**

How does the biology of HIV necessitate the need to treat HIV infections with multiple drugs?

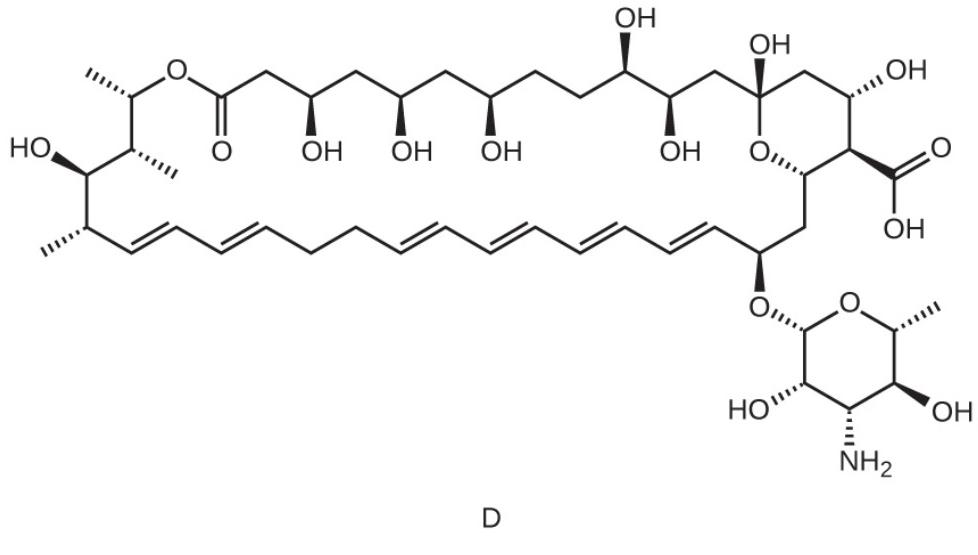
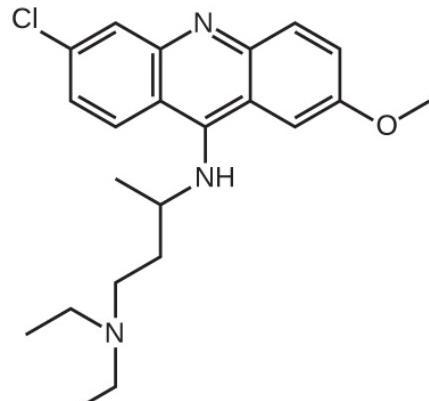
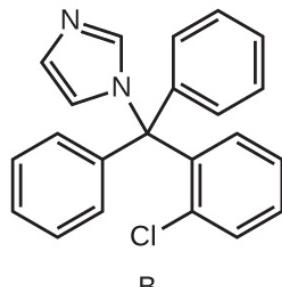
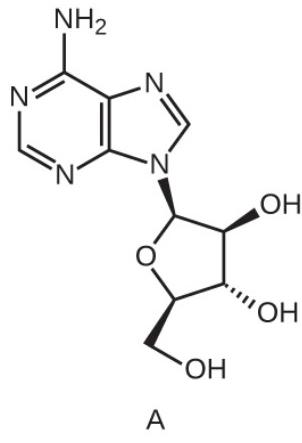
Exercise:**Problem:**

Niclosamide is insoluble and thus is not readily absorbed from the stomach into the bloodstream. How does the insolubility of niclosamide aid its effectiveness as a treatment for tapeworm infection?

Critical Thinking**Exercise:**

Problem:

Which of the following molecules is an example of a nucleoside analog?



Exercise:

Problem:

Why can't drugs used to treat influenza, like amantadines and neuraminidase inhibitors, be used to treat a wider variety of viral infections?

Drug Resistance

LEARNING OBJECTIVES

- Explain the concept of drug resistance
- Describe how microorganisms develop or acquire drug resistance
- Describe the different mechanisms of antimicrobial drug resistance

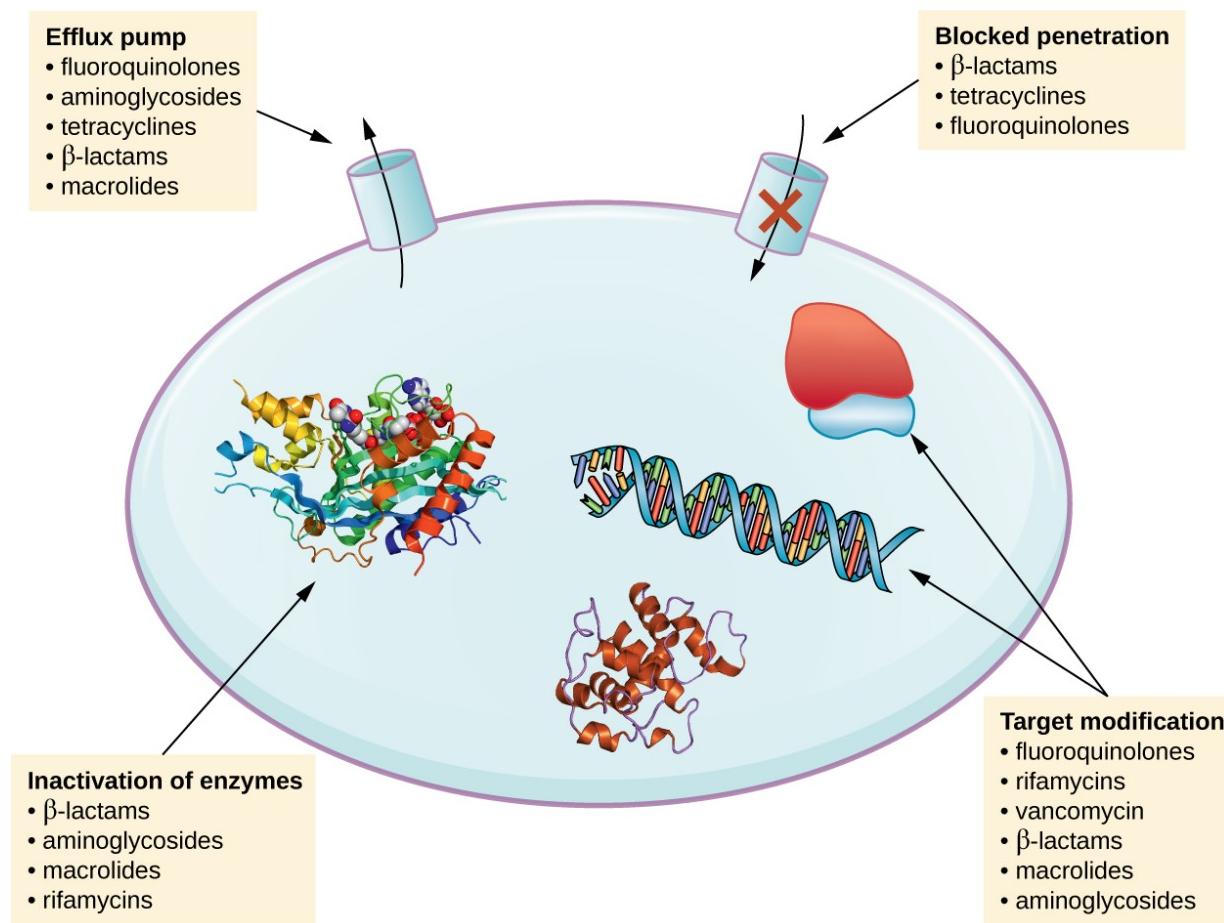
Antimicrobial resistance is not a new phenomenon. In nature, microbes are constantly evolving in order to overcome the antimicrobial compounds produced by other microorganisms. Human development of antimicrobial drugs and their widespread clinical use has simply provided another selective pressure that promotes further evolution. Several important factors can accelerate the evolution of **drug resistance**. These include the overuse and misuse of antimicrobials, inappropriate use of antimicrobials, subtherapeutic dosing, and patient noncompliance with the recommended course of treatment.

Exposure of a pathogen to an antimicrobial compound can select for chromosomal mutations conferring resistance, which can be transferred vertically to subsequent microbial generations and eventually become predominant in a microbial population that is repeatedly exposed to the antimicrobial. Alternatively, many genes responsible for drug resistance are found on plasmids or in transposons that can be transferred easily between microbes through horizontal gene transfer (see [How Asexual Prokaryotes Achieve Genetic Diversity](#)). Transposons also have the ability to move

resistance genes between plasmids and chromosomes to further promote the spread of resistance.

Mechanisms for Drug Resistance

There are several common mechanisms for drug resistance, which are summarized in [\[link\]](#). These mechanisms include enzymatic modification of the drug, modification of the antimicrobial target, and prevention of drug penetration or accumulation.



There are multiple strategies that microbes use to develop resistance to antimicrobial drugs. (Not shown: target overproduction, target mimicry, and enzymatic bypass). (credit: modification of work by Gerard D Wright)

Drug Modification or Inactivation

Resistance genes may code for enzymes that chemically modify an antimicrobial, thereby inactivating it, or destroy an antimicrobial through hydrolysis. Resistance to many types of antimicrobials occurs through this mechanism. For example, aminoglycoside resistance can occur through enzymatic transfer of chemical groups to the drug molecule, impairing the binding of the drug to its bacterial target. For β -lactams, bacterial resistance can involve the enzymatic hydrolysis of the β -lactam bond within the β -lactam ring of the drug molecule. Once the β -lactam bond is broken, the drug loses its antibacterial activity. This mechanism of resistance is mediated by β -lactamases, which are the most common mechanism of β -lactam resistance. Inactivation of rifampin commonly occurs through glycosylation, phosphorylation, or adenosine diphosphate (ADP) ribosylation, and resistance to macrolides and lincosamides can also occur due to enzymatic inactivation of the drug or modification.

Prevention of Cellular Uptake or Efflux

Microbes may develop resistance mechanisms that involve inhibiting the accumulation of an antimicrobial drug, which then prevents the drug from reaching its cellular target. This strategy is common among gram-negative pathogens and can involve changes in outer membrane lipid composition, porin channel selectivity, and/or porin channel concentrations. For example, a common mechanism of carbapenem resistance among *Pseudomonas aeruginosa* is to decrease the amount of its OprD porin, which is the primary portal of entry for carbapenems through the outer membrane of this pathogen. Additionally, many gram-positive and gram-negative pathogenic bacteria produce efflux pumps that actively transport an antimicrobial drug out of the cell and prevent the accumulation of drug to a level that would be antibacterial. For example, resistance to β -lactams, tetracyclines, and

fluoroquinolones commonly occurs through active efflux out of the cell, and it is rather common for a single efflux pump to have the ability to translocate multiple types of antimicrobials.

Target Modification

Because antimicrobial drugs have very specific targets, structural changes to those targets can prevent drug binding, rendering the drug ineffective. Through spontaneous mutations in the genes encoding antibacterial drug targets, bacteria have an evolutionary advantage that allows them to develop resistance to drugs. This mechanism of resistance development is quite common. Genetic changes impacting the active site of penicillin-binding proteins (PBPs) can inhibit the binding of β -lactam drugs and provide resistance to multiple drugs within this class. This mechanism is very common among strains of *Streptococcus pneumoniae*, which alter their own PBPs through genetic mechanisms. In contrast, strains of *Staphylococcus aureus* develop resistance to methicillin (MRSA) through the acquisition of a new low-affinity PBP, rather than structurally alter their existing PBPs. Not only does this new low-affinity PBP provide resistance to methicillin but it provides resistance to virtually all β -lactam drugs, with the exception of the newer fifth-generation cephalosporins designed specifically to kill MRSA. Other examples of this resistance strategy include alterations in

- ribosome subunits, providing resistance to macrolides, tetracyclines, and aminoglycosides;
- lipopolysaccharide (LPS) structure, providing resistance to polymyxins;
- RNA polymerase, providing resistance to rifampin;
- DNA gyrase, providing resistance to fluoroquinolones;
- metabolic enzymes, providing resistance to sulfa drugs, sulfones, and trimethoprim; and
- peptidoglycan subunit peptide chains, providing resistance to glycopeptides.

Target Overproduction or Enzymatic Bypass

When an antimicrobial drug functions as an antimetabolite, targeting a specific enzyme to inhibit its activity, there are additional ways that microbial resistance may occur. First, the microbe may overproduce the target enzyme such that there is a sufficient amount of antimicrobial-free enzyme to carry out the proper enzymatic reaction. Second, the bacterial cell may develop a bypass that circumvents the need for the functional target enzyme. Both of these strategies have been found as mechanisms of sulfonamide resistance. Vancomycin resistance among *S. aureus* has been shown to involve the decreased cross-linkage of peptide chains in the bacterial cell wall, which provides an increase in targets for vancomycin to bind to in the outer cell wall. Increased binding of vancomycin in the outer cell wall provides a blockage that prevents free drug molecules from penetrating to where they can block new cell wall synthesis.

Target Mimicry

A recently discovered mechanism of resistance called target mimicry involves the production of proteins that bind and sequester drugs, preventing the drugs from binding to their target. For example, *Mycobacterium tuberculosis* produces a protein with regular pentapeptide repeats that appears to mimic the structure of DNA. This protein binds fluoroquinolones, sequestering them and keeping them from binding to DNA, providing *M. tuberculosis* resistance to fluoroquinolones. Proteins that mimic the A-site of the bacterial ribosome have been found to contribute to aminoglycoside resistance as well.[\[footnote\]](#)
D.H. Fong, A.M. Berghuis. “Substrate Promiscuity of an Aminoglycoside Antibiotic Resistance Enzyme Via Target Mimicry.” *EMBO Journal* 21 no. 10 (2002):2323–2331.

Note:

- List several mechanisms for drug resistance.

Multidrug-Resistant Microbes and Cross Resistance

From a clinical perspective, our greatest concerns are **multidrug-resistant microbes (MDRs)** and cross resistance. MDRs are colloquially known as “superbugs” and carry one or more resistance mechanism(s), making them resistant to multiple antimicrobials. In **cross-resistance**, a single resistance mechanism confers resistance to multiple antimicrobial drugs. For example, having an efflux pump that can export multiple antimicrobial drugs is a common way for microbes to be resistant to multiple drugs by using a single resistance mechanism. In recent years, several clinically important superbugs have emerged, and the CDC reports that superbugs are responsible for more than 2 million infections in the US annually, resulting in at least 23,000 fatalities.[\[footnote\]](#) Several of the superbugs discussed in the following sections have been dubbed the ESKAPE pathogens. This acronym refers to the names of the pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.) but it is also fitting in that these pathogens are able to “escape” many conventional forms of antimicrobial therapy. As such, infections by ESKAPE pathogens can be difficult to treat and they cause a large number of nosocomial infections.

Centers for Disease Control and Prevention. “Antibiotic/Antimicrobial Resistance.” <http://www.cdc.gov/drugresistance/index.html>. Accessed June 2, 2016.

Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Methicillin, a semisynthetic penicillin, was designed to resist inactivation by β -lactamases. Unfortunately, soon after the introduction of methicillin to clinical practice, methicillin-resistant strains of *S. aureus* appeared and started to spread. The mechanism of resistance, acquisition of a new low-affinity PBP, provided *S. aureus* with resistance to all available β -lactams. Strains of **methicillin-resistant *S. aureus* (MRSA)** are widespread opportunistic pathogens and a particular concern for skin and other wound infections, but may also cause pneumonia and septicemia. Although

originally a problem in health-care settings (hospital-acquired MRSA [HA-MRSA]), MRSA infections are now also acquired through contact with contaminated members of the general public, called community-associated MRSA (CA-MRSA). Approximately one-third of the population carries *S. aureus* as a member of their normal nasal microbiota without illness, and about 6% of these strains are methicillin resistant.[\[footnote\]](#)[\[footnote\]](#)

A.S. Kalokhe et al. “Multidrug-Resistant Tuberculosis Drug Susceptibility and Molecular Diagnostic Testing: A Review of the Literature. *American Journal of the Medical Sciences* 345 no. 2 (2013):143–148.

Centers for Disease Control and Prevention. “Methicillin-Resistant *Staphylococcus aureus* (MRSA): General Information About MRSA in the Community.” <http://www.cdc.gov/mrsa/community/index.html>. Accessed June 2, 2016

Note:

Clavulanic Acid: Penicillin’s Little Helper

With the introduction of penicillin in the early 1940s, and its subsequent mass production, society began to think of antibiotics as miracle cures for a wide range of infectious diseases. Unfortunately, as early as 1945, penicillin resistance was first documented and started to spread. Greater than 90% of current *S. aureus* clinical isolates are resistant to penicillin.

[\[footnote\]](#)

F.D. Lowy. “Antimicrobial Resistance: The Example of *Staphylococcus aureus*.” *Journal of Clinical Investigation* 111 no. 9 (2003):1265–1273. Although developing new antimicrobial drugs is one solution to this problem, scientists have explored new approaches, including the development of compounds that inactivate resistance mechanisms. The development of clavulanic acid represents an early example of this strategy. Clavulanic acid is a molecule produced by the bacterium *Streptococcus claviger*. It contains a β-lactam ring, making it structurally similar to penicillin and other β-lactams, but shows no clinical effectiveness when administered on its own. Instead, clavulanic acid binds irreversibly within the active site of β-lactamases and prevents them from inactivating a coadministered penicillin.

Clavulanic acid was first developed in the 1970s and was mass marketed in combination with amoxicillin beginning in the 1980s under the brand name Augmentin. As is typically the case, resistance to the amoxicillin-clavulanic acid combination soon appeared. Resistance most commonly results from bacteria increasing production of their β -lactamase and overwhelming the inhibitory effects of clavulanic acid, mutating their β -lactamase so it is no longer inhibited by clavulanic acid, or from acquiring a new β -lactamase that is not inhibited by clavulanic acid. Despite increasing resistance concerns, clavulanic acid and related β -lactamase inhibitors (sulbactam and tazobactam) represent an important new strategy: the development of compounds that directly inhibit antimicrobial resistance-conferring enzymes.

Vancomycin-Resistant Enterococci and *Staphylococcus aureus*

Vancomycin is only effective against gram-positive organisms, and it is used to treat wound infections, septic infections, endocarditis, and meningitis that are caused by pathogens resistant to other antibiotics. It is considered one of the last lines of defense against such resistant infections, including MRSA. With the rise of antibiotic resistance in the 1970s and 1980s, vancomycin use increased, and it is not surprising that we saw the emergence and spread of **vancomycin-resistant enterococci (VRE)**, **vancomycin-resistant *S. aureus* (VRSA)**, and **vancomycin-intermediate *S. aureus* (VISA)**. The mechanism of vancomycin resistance among enterococci is target modification involving a structural change to the peptide component of the peptidoglycan subunits, preventing vancomycin from binding. These strains are typically spread among patients in clinical settings by contact with health-care workers and contaminated surfaces and medical equipment.

VISA and VRSA strains differ from each other in the mechanism of resistance and the degree of resistance each mechanism confers. VISA strains exhibit intermediate resistance, with a minimum inhibitory concentration (MIC) of 4–8 $\mu\text{g}/\text{mL}$, and the mechanism involves an increase in vancomycin targets. VISA strains decrease the crosslinking of

peptide chains in the cell wall, providing an increase in vancomycin targets that trap vancomycin in the outer cell wall. In contrast, VRSA strains acquire vancomycin resistance through horizontal transfer of resistance genes from VRE, an opportunity provided in individuals coinfecte^d with both VRE and MRSA. VRSA exhibit a higher level of resistance, with MICs of 16 µg/mL or higher.[\[footnote\]](#) In the case of all three types of vancomycin-resistant bacteria, rapid clinical identification is necessary so proper procedures to limit spread can be implemented. The oxazolidinones like linezolid are useful for the treatment of these vancomycin-resistant, opportunistic pathogens, as well as MRSA.

Centers for Disease Control and Prevention. “Healthcare-Associated Infections (HAI): General Information about VISA/VRSA.” http://www.cdc.gov/HAI/organisms/visa_vrsa/visa_vrsa.html. Accessed June 2, 2016.

Extended-Spectrum β -Lactamase–Producing Gram-Negative Pathogens

Gram-negative pathogens that produce **extended-spectrum β -lactamases (ESBLs)** show resistance well beyond just penicillins. The spectrum of β -lactams inactivated by ESBLs provides for resistance to all penicillins, cephalosporins, monobactams, and the β -lactamase-inhibitor combinations, but not the carbapenems. An even greater concern is that the genes encoding for ESBLs are usually found on mobile plasmids that also contain genes for resistance to other drug classes (e.g., fluoroquinolones, aminoglycosides, tetracyclines), and may be readily spread to other bacteria by horizontal gene transfer. These multidrug-resistant bacteria are members of the intestinal microbiota of some individuals, but they are also important causes of opportunistic infections in hospitalized patients, from whom they can be spread to other people.

Carbapenem-Resistant Gram-Negative Bacteria

The occurrence of **carbapenem-resistant Enterobacteriaceae (CRE)** and carbapenem resistance among other gram-negative bacteria (e.g., *P. aeruginosa*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia*) is a

growing health-care concern. These pathogens develop resistance to carbapenems through a variety of mechanisms, including production of carbapenemases (broad-spectrum β -lactamases that inactivate all β -lactams, including carbapenems), active efflux of carbapenems out of the cell, and/or prevention of carbapenem entry through porin channels. Similar to concerns with ESBLs, carbapenem-resistant, gram-negative pathogens are usually resistant to multiple classes of antibacterials, and some have even developed pan-resistance (resistance to all available antibacterials). Infections with carbapenem-resistant, gram-negative pathogens commonly occur in health-care settings through interaction with contaminated individuals or medical devices, or as a result of surgery.

Multidrug-Resistant *Mycobacterium tuberculosis*

The emergence of **multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB)** and **extensively drug-resistant *Mycobacterium tuberculosis* (XDR-TB)** is also of significant global concern. MDR-TB strains are resistant to both rifampin and isoniazid, the drug combination typically prescribed for treatment of tuberculosis. XDR-TB strains are additionally resistant to any fluoroquinolone and at least one of three other drugs (amikacin, kanamycin, or capreomycin) used as a second line of treatment, leaving these patients very few treatment options. Both types of pathogens are particularly problematic in immunocompromised persons, including those suffering from HIV infection. The development of resistance in these strains often results from the incorrect use of antimicrobials for tuberculosis treatment, selecting for resistance.

Note:

- How does drug resistance lead to superbugs?

Note:



To learn more about the [top 18 drug-resistant threats](#) to the US, visit the CDC's website.

Note:

Factory Farming and Drug Resistance

Although animal husbandry has long been a major part of agriculture in America, the rise of concentrated animal feeding operations (CAFOs) since the 1950s has brought about some new environmental issues, including the contamination of water and air with biological waste, and ethical issues regarding animal rights also are associated with growing animals in this way. Additionally, the increase in CAFOs involves the extensive use of antimicrobial drugs in raising livestock. Antimicrobials are used to prevent the development of infectious disease in the close quarters of CAFOs; however, the majority of antimicrobials used in factory farming are for the promotion of growth—in other words, to grow larger animals.

The mechanism underlying this enhanced growth remains unclear. These antibiotics may not necessarily be the same as those used clinically for humans, but they are structurally related to drugs used for humans. As a result, use of antimicrobial drugs in animals can select for antimicrobial resistance, with these resistant bacteria becoming cross-resistant to drugs typically used in humans. For example, tylosin use in animals appears to select for bacteria also cross-resistant to other macrolides, including erythromycin, commonly used in humans.

Concentrations of the drug-resistant bacterial strains generated by CAFOs become increased in water and soil surrounding these farms. If not directly pathogenic in humans, these resistant bacteria may serve as a reservoir of mobile genetic elements that can then pass resistance genes to human

pathogens. Fortunately, the cooking process typically inactivates any antimicrobials remaining in meat, so humans typically are not directly ingesting these drugs. Nevertheless, many people are calling for more judicious use of these drugs, perhaps charging farmers user fees to reduce indiscriminate use. In fact, in 2012, the FDA published guidelines for farmers who voluntarily phase out the use of antimicrobial drugs except under veterinary supervision and when necessary to ensure animal health. Although following the guidelines is voluntary at this time, the FDA does recommend what it calls “judicious” use of antimicrobial drugs in food-producing animals in an effort to decrease antimicrobial resistance.

Key Concepts and Summary

- **Antimicrobial resistance** is on the rise and is the result of selection of drug-resistant strains in clinical environments, the overuse and misuse of antibacterials, the use of subtherapeutic doses of antibacterial drugs, and poor patient compliance with antibacterial drug therapies.
- Drug resistance genes are often carried on plasmids or in transposons that can undergo vertical transfer easily and between microbes through horizontal gene transfer.
- Common modes of antimicrobial drug resistance include drug modification or inactivation, prevention of cellular uptake or efflux, target modification, target overproduction or enzymatic bypass, and target mimicry.
- Problematic microbial strains showing extensive antimicrobial resistance are emerging; many of these strains can reside as members of the normal microbiota in individuals but also can cause opportunistic infection. The transmission of many of these highly resistant microbial strains often occurs in clinical settings, but can also be community-acquired.

Short Answer

Exercise:

Problem:

Why does the length of time of antimicrobial treatment for tuberculosis contribute to the rise of resistant strains?

Exercise:

Problem:

What is the difference between multidrug resistance and cross-resistance?

Testing the Effectiveness of Antimicrobials

LEARNING OBJECTIVES

- Describe how the Kirby-Bauer disk diffusion test determines the susceptibility of a microbe to an antibacterial drug.
- Explain the significance of the minimal inhibitory concentration and the minimal bactericidal concentration relative to the effectiveness of an antimicrobial drug.

Testing the effectiveness of antimicrobial drugs against specific organisms is important in identifying their spectrum of activity and the therapeutic dosage. This type of test, generally described as antimicrobial susceptibility testing (AST), is commonly performed in a clinical laboratory. In this section, we will discuss common methods of testing the effectiveness of antimicrobials.

The Kirby-Bauer Disk Diffusion Test

The **Kirby-Bauer disk diffusion test** has long been used as a starting point for determining the susceptibility of specific microbes to various antimicrobial drugs. The Kirby-Bauer assay starts with a Mueller-Hinton agar plate on which a confluent lawn is inoculated with a patient's isolated bacterial pathogen. Filter paper disks impregnated with known amounts of antibacterial drugs to be tested are then placed on the agar plate. As the bacterial inoculum grows, antibiotic diffuses from the circular disk into the agar and interacts with the growing bacteria. Antibacterial activity is observed as a clear circular **zone of inhibition** around the drug-impregnated disk, similar to the disk-diffusion assay depicted in [\[link\]](#). The diameter of

the zone of inhibition, measured in millimeters and compared to a standardized chart, determines the susceptibility or resistance of the bacterial pathogen to the drug.

There are multiple factors that determine the size of a zone of inhibition in this assay, including drug solubility, rate of drug diffusion through agar, the thickness of the agar medium, and the drug concentration impregnated into the disk. Due to a lack of standardization of these factors, interpretation of the Kirby-Bauer disk diffusion assay provides only limited information on susceptibility and resistance to the drugs tested. The assay cannot distinguish between bacteriostatic and bactericidal activities, and differences in zone sizes cannot be used to compare drug potencies or efficacies. Comparison of zone sizes to a standardized chart will only provide information on the antibacterials to which a bacterial pathogen is susceptible or resistant.

Note:

- How does one use the information from a Kirby-Bauer assay to predict the therapeutic effectiveness of an antimicrobial drug in a patient?

Note:

Antibiograms: Taking Some of the Guesswork Out of Prescriptions

Unfortunately, infectious diseases don't take a time-out for lab work. As a result, physicians rarely have the luxury of conducting susceptibility testing before they write a prescription. Instead, they rely primarily on the empirical evidence (i.e., the signs and symptoms of disease) and their professional experience to make an educated guess as to the diagnosis, causative agent(s), and drug most likely to be effective. This approach allows treatment to begin sooner so the patient does not have to wait for lab test results. In many cases, the prescription is effective; however, in an age of increased antimicrobial resistance, it is becoming increasingly more

difficult to select the most appropriate empiric therapy. Selecting an inappropriate empiric therapy not only puts the patient at risk but may promote greater resistance to the drug prescribed.

Recently, studies have shown that antibiograms are useful tools in the decision-making process of selecting appropriate empiric therapy. An **antibiogram** is a compilation of local antibiotic susceptibility data broken down by bacterial pathogen. In a November 2014 study published in the journal *Infection Control and Hospital Epidemiology*, researchers determined that 85% of the prescriptions ordered in skilled nursing facilities were decided upon empirically, but only 35% of those prescriptions were deemed appropriate when compared with the eventual pathogen identification and susceptibility profile obtained from the clinical laboratory. However, in one nursing facility where use of antibiograms was implemented to direct selection of empiric therapy, appropriateness of empiric therapy increased from 32% before antibiogram implementation to 45% after implementation of antibiograms.[\[footnote\]](#) Although these data are preliminary, they do suggest that health-care facilities can reduce the number of inappropriate prescriptions by using antibiograms to select empiric therapy, thus benefiting patients and minimizing opportunities for antimicrobial resistance to develop.

J.P. Furuno et al. “Using Antibiograms to Improve Antibiotic Prescribing in Skilled Nursing Facilities.” *Infection Control and Hospital Epidemiology* 35 no. Suppl S3 (2014):S56–61.

Note:



Visit this website to view an [interactive antibiogram](#) provided by Stanford University.

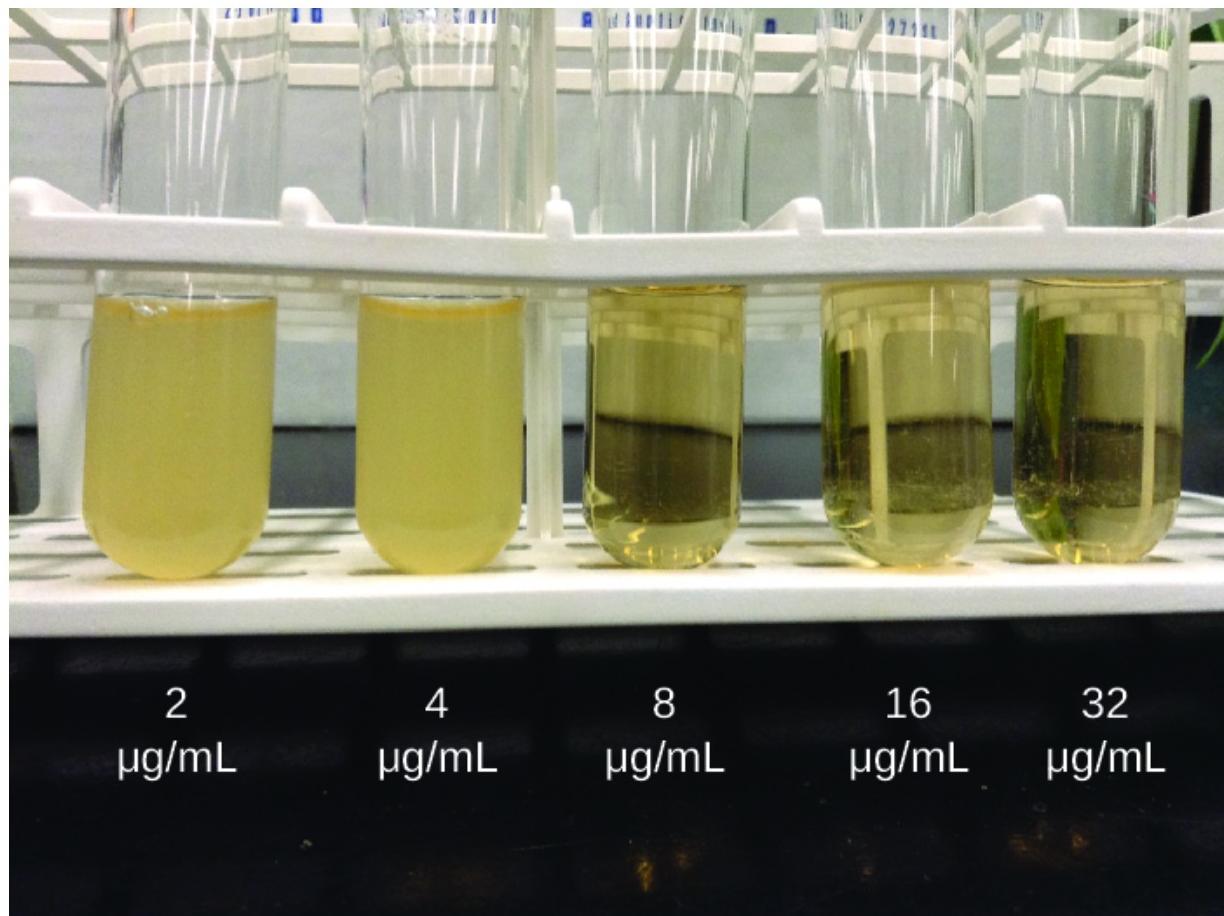
Dilution Tests

As discussed, the limitations of the Kirby-Bauer disk diffusion test do not allow for a direct comparison of antibacterial potencies to guide selection of the best therapeutic choice. However, antibacterial dilution tests can be used to determine a particular drug's **minimal inhibitory concentration (MIC)**, the lowest concentration of drug that inhibits visible bacterial growth, and **minimal bactericidal concentration (MBC)**, the lowest drug concentration that kills ≥99.9% of the starting inoculum. Determining these concentrations helps identify the correct drug for a particular pathogen. For the macrobroth dilution assay, a dilution series of the drug in broth is made in test tubes and the same number of cells of a test bacterial strain is added to each tube ([\[link\]](#)). The MIC is determined by examining the tubes to find the lowest drug concentration that inhibits visible growth; this is observed as turbidity (cloudiness) in the broth. Tubes with no visible growth are then inoculated onto agar media without antibiotic to determine the MBC. Generally, serum levels of an antibacterial should be at least three to five times above the MIC for treatment of an infection.

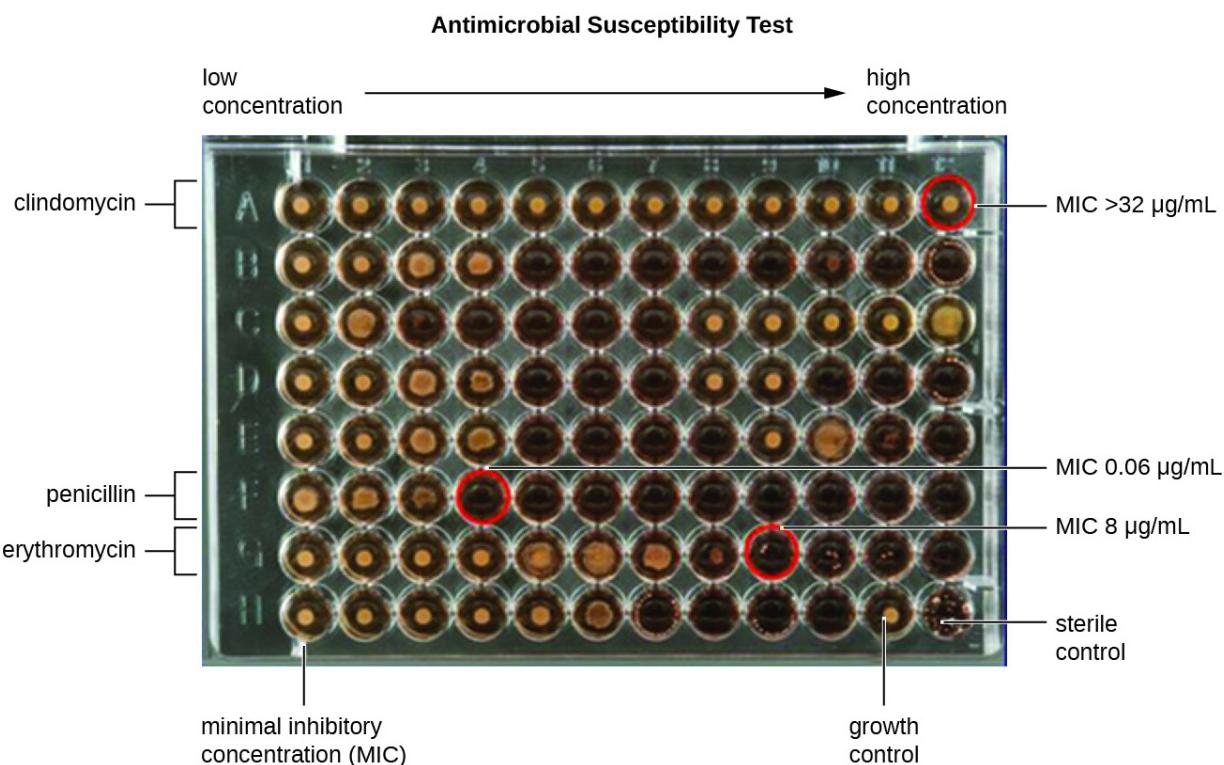
The MIC assay can also be performed using 96-well microdilution trays, which allow for the use of small volumes and automated dispensing devices, as well as the testing of multiple antimicrobials and/or microorganisms in one tray ([\[link\]](#)). MICs are interpreted as the lowest concentration that inhibits visible growth, the same as for the macrobroth dilution in test tubes. Growth may also be interpreted visually or by using a spectrophotometer or similar device to detect turbidity or a color change if an appropriate biochemical substrate that changes color in the presence of bacterial growth is also included in each well.

The **Etest** is an alternative method used to determine MIC, and is a combination of the Kirby-Bauer disk diffusion test and dilution methods. Similar to the Kirby-Bauer assay, a confluent lawn of a bacterial isolate is inoculated onto the surface of an agar plate. Rather than using circular disks

impregnated with one concentration of drug, however, commercially available plastic strips that contain a gradient of an antibacterial are placed on the surface of the inoculated agar plate ([\[link\]](#)). As the bacterial inoculum grows, antibiotic diffuses from the plastic strips into the agar and interacts with the bacterial cells. Because the rate of drug diffusion is directly related to concentration, an elliptical zone of inhibition is observed with the Etest drug gradient, rather than a circular zone of inhibition observed with the Kirby-Bauer assay. To interpret the results, the intersection of the elliptical zone with the gradient on the drug-containing strip indicates the MIC. Because multiple strips containing different antimicrobials can be placed on the same plate, the MIC of multiple antimicrobials can be determined concurrently and directly compared. However, unlike the macrobroth and microbroth dilution methods, the MBC cannot be determined with the Etest.

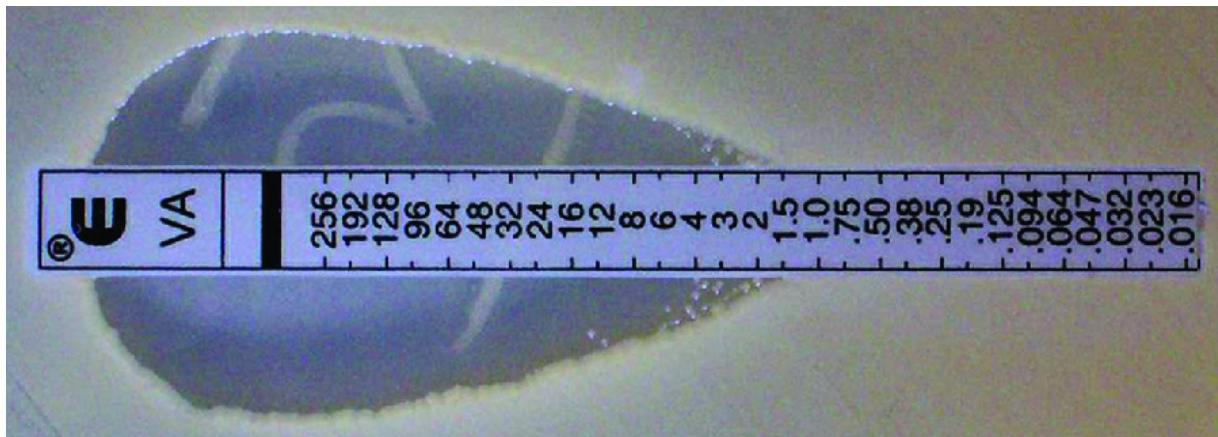


In a dilution test, the lowest dilution that inhibits turbidity (cloudiness) is the MIC. In this example, the MIC is 8 µg/mL. Broth from samples without turbidity can be inoculated onto plates lacking the antimicrobial drug. The lowest dilution that kills ≥99.9% of the starting inoculum is observed on the plates is the MBC. (credit: modification of work by Suzanne Wakim)



A microdilution tray can also be used to determine MICs of multiple antimicrobial drugs in a single assay. In this example, the drug concentrations increase from left to right and the rows with clindamycin, penicillin, and erythromycin have been indicated to the left of the plate. For penicillin and erythromycin, the lowest concentrations that inhibited visible growth are indicated by red circles and were 0.06 µg/mL for penicillin and 8 µg/mL for erythromycin. For clindamycin, visible bacterial growth was observed at every concentration up to 32 µg/mL and the MIC is interpreted as >32

$\mu\text{g/mL}$. (credit: modification of work by Centers for Disease Control and Prevention)



The Etest can be used to determine the MIC of an antibiotic. In this Etest, vancomycin is shown to have a MIC of $1.5 \mu\text{g/mL}$ against *Staphylococcus aureus*.

Note:

- Compare and contrast MIC and MBC.

Note:

Resolution

Marisa's UTI was likely caused by the catheterizations she had in Vietnam. Most bacteria that cause UTIs are members of the normal gut microbiota, but they can cause infections when introduced to the urinary tract, as might

have occurred when the catheter was inserted. Alternatively, if the catheter itself was not sterile, bacteria on its surface could have been introduced into Marisa's body. The antimicrobial therapy Marisa received in Cambodia may also have been a complicating factor because it may have selected for antimicrobial-resistant strains already present in her body. These bacteria would have already contained genes for antimicrobial resistance, either acquired by spontaneous mutation or through horizontal gene transfer, and, therefore, had the best evolutionary advantage for adaptation and growth in the presence of the antimicrobial therapy. As a result, one of these resistant strains may have been subsequently introduced into her urinary tract.

Laboratory testing at the CDC confirmed that the strain of *Klebsiella pneumoniae* from Marisa's urine sample was positive for the presence of NDM, a very active carbapenemase that is beginning to emerge as a new problem in antimicrobial resistance. While NDM-positive strains are resistant to a wide range of antimicrobials, they have shown susceptibility to tigecycline (structurally related to tetracycline) and the polymyxins B and E (colistin).

To prevent her infection from spreading, Marisa was isolated from the other patients in a separate room. All hospital staff interacting with her were advised to follow strict protocols to prevent surface and equipment contamination. This would include especially stringent hand hygiene practices and careful disinfection of all items coming into contact with her. Marisa's infection finally responded to tigecycline and eventually cleared. She was discharged a few weeks after admission, and a follow-up stool sample showed her stool to be free of NDM-containing *K. pneumoniae*, meaning that she was no longer harboring the highly resistant bacterium.

Go back to the [previous](#) Clinical Focus box.

Key Concepts and Summary

- The **Kirby-Bauer disk diffusion** test helps determine the susceptibility of a microorganism to various antimicrobial drugs. However, the **zones of inhibition** measured must be correlated to known standards to determine susceptibility and resistance, and do not

provide information on bactericidal versus bacteriostatic activity, or allow for direct comparison of drug potencies.

- Antibiograms are useful for monitoring local trends in antimicrobial resistance/susceptibility and for directing appropriate selection of empiric antibacterial therapy.
- There are several laboratory methods available for determining the **minimum inhibitory concentration (MIC)** of an antimicrobial drug against a specific microbe. The **minimal bactericidal concentration (MBC)** can also be determined, typically as a follow-up experiment to MIC determination using the tube dilution method.

Multiple Choice

Exercise:

Problem:

In the Kirby-Bauer disk diffusion test, the _____ of the zone of inhibition is measured and used for interpretation.

- A. diameter
- B. microbial population
- C. circumference
- D. depth

Solution:

A

Exercise:

Problem:

Which of the following techniques cannot be used to determine the minimum inhibitory concentration of an antimicrobial drug against a particular microbe?

- A. Etest

- B. microbroth dilution test
 - C. Kirby-Bauer disk diffusion test
 - D. macrobroth dilution test
-

Solution:

C

Exercise:

Problem:

The utility of an antibiogram is that it shows antimicrobial susceptibility trends

- A. over a large geographic area.
 - B. for an individual patient.
 - C. in research laboratory strains.
 - D. in a localized population.
-

Solution:

D

Fill in the Blank

Exercise:

Problem:

The method that can determine the MICs of multiple antimicrobial drugs against a microbial strain using a single agar plate is called the _____.

Solution:

Etest

True/False

Exercise:

Problem:

If drug A produces a larger zone of inhibition than drug B on the Kirby-Bauer disk diffusion test, drug A should always be prescribed.

Solution:

false

Short Answer

Exercise:

Problem:

How is the information from a Kirby-Bauer disk diffusion test used for the recommendation of the clinical use of an antimicrobial drug?

Exercise:

Problem: What is the difference between MIC and MBC?

Critical Thinking

Exercise:

Problem: Can an Etest be used to find the MBC of a drug? Explain.

Current Strategies for Antimicrobial Discovery

LEARNING OBJECTIVES

- Describe the methods and strategies used for discovery of new antimicrobial agents.

With the continued evolution and spread of antimicrobial resistance, and now the identification of pan-resistant bacterial pathogens, the search for new antimicrobials is essential for preventing the postantibiotic era.

Although development of more effective semisynthetic derivatives is one strategy, resistance to them develops rapidly because bacterial pathogens are already resistant to earlier-generation drugs in the family and can easily mutate and develop resistance to the new semisynthetic drugs. Today, scientists continue to hunt for new antimicrobial compounds and explore new avenues of antimicrobial discovery and synthesis. They check large numbers of soils and microbial products for antimicrobial activity by using high-throughput screening methods, which use automation to test large numbers of samples simultaneously. The recent development of the iChip[[footnote](#)] allows researchers to investigate the antimicrobial-producing capabilities of soil microbes that are difficult to grow by standard cultivation techniques in the laboratory. Rather than grow the microbes in the laboratory, they are grown *in situ*—right in the soil. Use of the iChip has resulted in the discovery of teixobactin, a novel antimicrobial from Mount Ararat, Turkey. Teixobactin targets two distinct steps in gram-positive cell wall synthesis and for which antimicrobial resistance appears not yet to have evolved.

L. Losee et al. “A New Antibiotic Kills Pathogens Without Detectable Resistance.” *Nature* 517 no. 7535 (2015):455–459.

Although soils have been widely examined, other environmental niches have not been tested as fully. Since 70% of the earth is covered with water, marine environments could be mined more fully for the presence of antimicrobial-producing microbes. In addition, researchers are using combinatorial chemistry, a method for making a very large number of related compounds from simple precursors, and testing them for antimicrobial activity. An additional strategy that needs to be explored further is the development of compounds that inhibit resistance mechanisms and restore the activity of older drugs, such as the strategy described earlier for β -lactamase inhibitors like clavulanic acid. Finally, developing inhibitors of virulence factor production and function could be a very important avenue. Although this strategy would not be directly antibacterial, drugs that slow the progression of an infection could provide an advantage for the immune system and could be used successfully in combination with antimicrobial drugs.

Note:

- What are new sources and strategies for developing drugs to fight infectious diseases?

Note:**The (Free?) Market for New Antimicrobials**

There used to be plenty of antimicrobial drugs on the market to treat infectious diseases. However, the spread of antimicrobial resistance has created a need for new antibiotics to replace those that are no longer as effective as they once were. Unfortunately, pharmaceutical companies are not particularly motivated to fill this need. As of 2009, all but five pharmaceutical companies had moved away from antimicrobial drug development.[\[footnote\]](#) As a result, the number of FDA approvals of new antimicrobials has fallen drastically in recent decades ([\[link\]](#)).

H.W. Boucher et al. “Bad Bugs, No Drugs: No ESKAPE! An Update from the Infectious Diseases Society of America.” *Clinical Infectious Diseases*

48 no. 1 (2009):1–12.

Given that demand usually encourages supply, one might expect pharmaceutical companies to be rushing to get back in the business of developing new antibiotics. But developing new drugs is a lengthy process and requires large investments in research and development.

Pharmaceutical companies can typically get a higher return on their investment by developing products for chronic, nonmicrobial diseases like diabetes; such drugs must be taken for life, and therefore generate more long-term revenue than an antibiotic that does its job in a week or two. But what will happen when drugs like vancomycin, a superantimicrobial reserved for use as a last resort, begin to lose their effectiveness against ever more drug-resistant superbugs? Will drug companies wait until all antibiotics have become useless before beginning to look for new ones?

Recently, it has been suggested that large pharmaceutical companies should be given financial incentives to pursue such research. In September 2014, the White House released an executive order entitled “Combating Antibiotic Resistant Bacteria,” calling upon various government agencies and the private sector to work together to “accelerate basic and applied research and development for new antimicrobials, other therapeutics, and vaccines.”[\[footnote\]](#) As a result, as of March 2015, President Obama’s proposed fiscal year 2016 budget doubled the amount of federal funding to \$1.2 billion for “combating and preventing antibiotic resistance,” which includes money for antimicrobial research and development.[\[footnote\]](#)

Similar suggestions have also been made on a global scale. In December 2014, a report chaired by former Goldman Sachs economist Jim O’Neill was published in *The Review on Antimicrobial Resistance*.[\[footnote\]](#)

The White House. *National Action Plan for Combating Antibiotic-Resistant Bacteria*. Washington, DC: The White House, 2015.

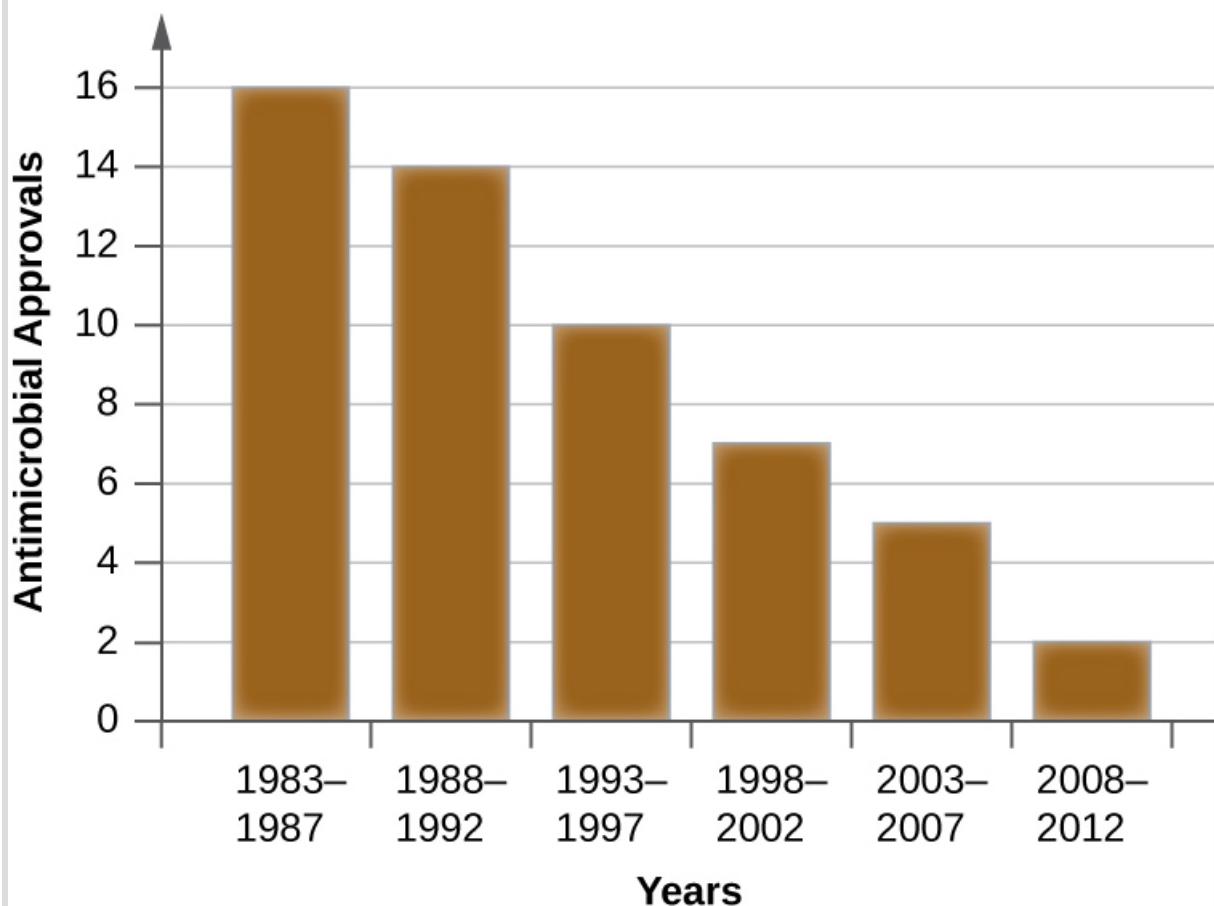
White House Office of the Press Secretary. “Fact Sheet: Obama Administration Releases National Action Plan to Combat Antibiotic-Resistant Bacteria.” March 27, 2015. <https://www.whitehouse.gov/the-press-office/2015/03/27/fact-sheet-obama-administration-releases-national-action-plan-combat-ant>

Review on Antimicrobial Resistance. <http://amr-review.org>. Accessed June 1, 2016.

These developments reflect the growing belief that for-profit pharmaceutical companies must be subsidized to encourage development

of new antimicrobials. But some ask whether pharmaceutical development should be motivated by profit at all. Given that millions of lives may hang in the balance, some might argue that drug companies have an ethical obligation to devote their research and development efforts to high-utility drugs, as opposed to highly profitable ones. Yet this obligation conflicts with the fundamental goals of a for-profit company. Are government subsidies enough to ensure that drug companies make the public interest a priority, or should government agencies assume responsibility for developing critical drugs that may have little or no return on investment?

New Antimicrobials Approved by FDA, 1983–2012



In recent decades, approvals of new antimicrobials by the FDA have steadily fallen. In the five-year period from 1983–1987, 16 new antimicrobial drugs were approved, compared to just two from 2008–2012.

Note:

To further examine the scope of the problem, view [this](#) video.

To [learn more](#) about the history of antimicrobial drug discovery, visit Michigan State University's Antimicrobial Resistance Learning Site.

Key Concepts and Summary

- Current research into the development of antimicrobial drugs involves the use of high-throughput screening and combinatorial chemistry technologies.
- New technologies are being developed to discover novel antibiotics from soil microorganisms that cannot be cultured by standard laboratory methods.
- Additional strategies include searching for antibiotics from sources other than soil, identifying new antibacterial targets, using combinatorial chemistry to develop novel drugs, developing drugs that inhibit resistance mechanisms, and developing drugs that target virulence factors and hold infections in check.

Critical Thinking

Exercise:

Problem:

Who should be responsible for discovering and developing new antibiotics? Support your answer with reasoning.

Microbial Pathogenesis - Introduction

class="introduction"

Although medical professionals rely heavily on signs and symptoms to diagnose disease and prescribe treatment, many diseases can produce similar signs and symptoms.

(credit left:
modification
of work by
U.S. Navy)



Jane woke up one spring morning feeling not quite herself. Her throat felt a bit dry and she was sniffling. She wondered why she felt so lousy. Was it because of a change in the weather? The pollen count? Was she coming down with something? Did she catch a bug from her coworker who sneezed on her in the elevator yesterday?

The signs and symptoms we associate with illness can have many different causes. Sometimes they are the direct result of a pathogenic infection, but in other cases they result from a response by our immune system to a pathogen or another perceived threat. For example, in response to certain pathogens, the immune system may release pyrogens, chemicals that cause the body temperature to rise, resulting in a fever. This response creates a less-than-favorable environment for the pathogen, but it also makes us feel sick.

Medical professionals rely heavily on analysis of signs and symptoms to determine the cause of an ailment and prescribe treatment. In some cases, signs and symptoms alone are enough to correctly identify the causative agent of a disease, but since few diseases produce truly unique symptoms, it is often necessary to confirm the identity of the infectious agent by other direct and indirect diagnostic methods.

Characteristics of Infectious Disease

LEARNING OBJECTIVES

- Distinguish between signs and symptoms of disease
- Explain the difference between a communicable disease and a noncommunicable disease
- Compare different types of infectious diseases, including iatrogenic, nosocomial, and zoonotic diseases
- Identify and describe the stages of an acute infectious disease in terms of number of pathogens present and severity of signs and symptoms

A **disease** is any condition in which the normal structure or functions of the body are damaged or impaired. Physical injuries or disabilities are not classified as disease, but there can be several causes for disease, including infection by a pathogen, genetics (as in many cancers or deficiencies), noninfectious environmental causes, or inappropriate immune responses. Our focus in this chapter will be on infectious diseases, although when diagnosing infectious diseases, it is always important to consider possible noninfectious causes.

Signs and Symptoms of Disease

An **infection** is the successful colonization of a host by a microorganism. Infections can lead to disease, which causes signs and symptoms resulting in a deviation from the normal structure or functioning of the host. Microorganisms that can cause disease are known as pathogens.

The **signs** of disease are objective and measurable, and can be directly observed by a clinician. Vital signs, which are used to measure the body's basic functions, include body temperature (normally 37 °C [98.6 °F]), heart rate (normally 60–100 beats per minute), breathing rate (normally 12–18 breaths per minute), and blood pressure (normally between 90/60 and 120/80 mm Hg). Changes in any of the body's vital signs may be indicative of disease. For example, having a fever (a body temperature significantly higher than 37 °C or 98.6 °F) is a sign of disease because it can be measured.

In addition to changes in vital signs, other observable conditions may be considered signs of disease. For example, the presence of antibodies in a patient's serum (the liquid portion of blood that lacks clotting factors) can be observed and measured through blood tests and, therefore, can be considered a sign. However, it is important to note that the presence of antibodies is not always a sign of an active disease. Antibodies can remain in the body long after an infection has resolved; also, they may develop in response to a pathogen that is in the body but not currently causing disease.

Unlike signs, **symptoms** of disease are subjective. Symptoms are felt or experienced by the patient, but they cannot be clinically confirmed or objectively measured. Examples of symptoms include nausea, loss of appetite, and pain. Such symptoms are important to consider when diagnosing disease, but they are subject to memory bias and are difficult to measure precisely. Some clinicians attempt to quantify symptoms by asking patients to assign a numerical value to their symptoms. For example, the Wong-Baker Faces pain-rating scale asks patients to rate their pain on a scale of 0–10. An alternative method of quantifying pain is measuring skin conductance fluctuations. These fluctuations reflect sweating due to skin sympathetic nerve activity resulting from the stressor of pain.[\[footnote\]](#)

F. Savino et al. “Pain Assessment in Children Undergoing Venipuncture: The Wong–Baker Faces Scale Versus Skin Conductance Fluctuations.” *PeerJ* 1 (2013):e37; <https://peerj.com/articles/37/>

A specific group of signs and symptoms characteristic of a particular disease is called a **syndrome**. Many syndromes are named using a nomenclature based on signs and symptoms or the location of the disease.

[\[link\]](#) lists some of the prefixes and suffixes commonly used in naming syndromes.

Nomenclature of Symptoms		
Affix	Meaning	Example
cyto-	cell	cytopenia: reduction in the number of blood cells
hepat-	of the liver	hepatitis: inflammation of the liver
-pathy	disease	neuropathy: a disease affecting nerves
-emia	of the blood	bacteremia: presence of bacteria in blood
-itis	inflammation	colitis: inflammation of the colon
-lysis	destruction	hemolysis: destruction of red blood cells
-oma	tumor	lymphoma: cancer of the lymphatic system
-osis	diseased or abnormal condition	leukocytosis: abnormally high number of white blood cells
-derma	of the skin	keratoderma: a thickening of the skin

Clinicians must rely on signs and on asking questions about symptoms, medical history, and the patient's recent activities to identify a particular disease and the potential causative agent. Diagnosis is complicated by the fact that different microorganisms can cause similar signs and symptoms in a patient. For example, an individual presenting with symptoms of diarrhea may have been infected by one of a wide variety of pathogenic microorganisms. Bacterial pathogens associated with diarrheal disease include *Vibrio cholerae*, *Listeria monocytogenes*, *Campylobacter jejuni*, and enteropathogenic *Escherichia coli* (EPEC). Viral pathogens associated with diarrheal disease include norovirus and rotavirus. Parasitic pathogens associated with diarrhea include *Giardia lamblia* and *Cryptosporidium parvum*. Likewise, fever is indicative of many types of infection, from the common cold to the deadly Ebola hemorrhagic fever.

Finally, some diseases may be **asymptomatic** or **subclinical**, meaning they do not present any noticeable signs or symptoms. For example, most individual infected with herpes simplex virus remain asymptomatic and are unaware that they have been infected.

Note:

- Explain the difference between signs and symptoms.

Classifications of Disease

The World Health Organization's (WHO) International Classification of Diseases (ICD) is used in clinical fields to classify diseases and monitor morbidity (the number of cases of a disease) and mortality (the number of deaths due to a disease). In this section, we will introduce terminology used by the ICD (and in health-care professions in general) to describe and categorize various types of disease.

An **infectious disease** is any disease caused by the direct effect of a pathogen. A pathogen may be cellular (bacteria, parasites, and fungi) or acellular (viruses, viroids, and prions). Some infectious diseases are also **communicable**, meaning they are capable of being spread from person to person through either direct or indirect mechanisms. Some infectious communicable diseases are also considered **contagious** diseases, meaning they are easily spread from person to person. Not all contagious diseases are equally so; the degree to which a disease is contagious usually depends on how the pathogen is transmitted. For example, measles is a highly contagious viral disease that can be transmitted when an infected person coughs or sneezes and an uninfected person breathes in droplets containing the virus. Gonorrhea is not as contagious as measles because transmission of the pathogen (*Neisseria gonorrhoeae*) requires close intimate contact (usually sexual) between an infected person and an uninfected person.

Diseases that are contracted as the result of a medical procedure are known as **iatrogenic diseases**. Iatrogenic diseases can occur after procedures involving wound treatments, catheterization, or surgery if the wound or surgical site becomes contaminated. For example, an individual treated for a skin wound might acquire necrotizing fasciitis (an aggressive, “flesh-eating” disease) if bandages or other dressings became contaminated by *Clostridium perfringens* or one of several other bacteria that can cause this condition.

Diseases acquired in hospital settings are known as **nosocomial diseases**. Several factors contribute to the prevalence and severity of nosocomial diseases. First, sick patients bring numerous pathogens into hospitals, and some of these pathogens can be transmitted easily via improperly sterilized medical equipment, bed sheets, call buttons, door handles, or by clinicians, nurses, or therapists who do not wash their hands before touching a patient. Second, many hospital patients have weakened immune systems, making them more susceptible to infections. Compounding this, the prevalence of antibiotics in hospital settings can select for drug-resistant bacteria that can cause very serious infections that are difficult to treat.

Certain infectious diseases are not transmitted between humans directly but can be transmitted from animals to humans. Such a disease is called

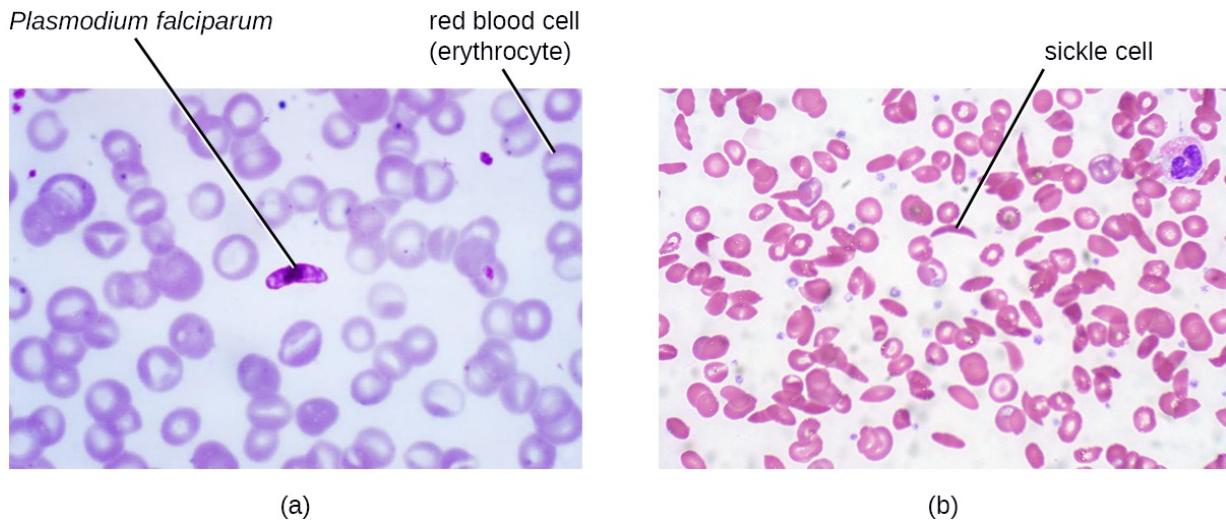
zoonotic disease (or **zoonosis**). According to WHO, a zoonosis is a disease that occurs when a pathogen is transferred from a vertebrate animal to a human; however, sometimes the term is defined more broadly to include diseases transmitted by all animals (including invertebrates). For example, rabies is a viral zoonotic disease spread from animals to humans through bites and contact with infected saliva. Many other zoonotic diseases rely on insects or other arthropods for transmission. Examples include yellow fever (transmitted through the bite of mosquitoes infected with yellow fever virus) and Rocky Mountain spotted fever (transmitted through the bite of ticks infected with *Rickettsia rickettsii*).

In contrast to communicable infectious diseases, a **noncommunicable** infectious disease is not spread from one person to another. One example is tetanus, caused by *Clostridium tetani*, a bacterium that produces endospores that can survive in the soil for many years. This disease is typically only transmitted through contact with a skin wound; it cannot be passed from an infected person to another person. Similarly, Legionnaires disease is caused by *Legionella pneumophila*, a bacterium that lives within amoebae in moist locations like water-cooling towers. An individual may contract Legionnaires disease via contact with the contaminated water, but once infected, the individual cannot pass the pathogen to other individuals.

In addition to the wide variety of noncommunicable infectious diseases, **noninfectious diseases** (those not caused by pathogens) are an important cause of morbidity and mortality worldwide. Noninfectious diseases can be caused by a wide variety factors, including genetics, the environment, or immune system dysfunction, to name a few. For example, sickle cell anemia is an inherited disease caused by a genetic mutation that can be passed from parent to offspring ([\[link\]](#)). Other types of noninfectious diseases are listed in [\[link\]](#).

Types of Noninfectious Diseases

Types of Noninfectious Diseases	Definition	Example
Type	Definition	Example
Inherited	A genetic disease	Sickle cell anemia
Congenital	Disease that is present at or before birth	Down syndrome
Degenerative	Progressive, irreversible loss of function	Parkinson disease (affecting central nervous system)
Nutritional deficiency	Impaired body function due to lack of nutrients	Scurvy (vitamin C deficiency)
Endocrine	Disease involving malfunction of glands that release hormones to regulate body functions	Hypothyroidism – thyroid does not produce enough thyroid hormone, which is important for metabolism
Neoplastic	Abnormal growth (benign or malignant)	Some forms of cancer
Idiopathic	Disease for which the cause is unknown	Idiopathic juxtafoveal retinal telangiectasia (dilated, twisted blood vessels in the retina of the eye)



Blood smears showing two diseases of the blood. (a) Malaria is an infectious, zoonotic disease caused by the protozoan pathogen *Plasmodium falciparum* (shown here) and several other species of the genus *Plasmodium*. It is transmitted by mosquitoes to humans. (b) Sickle cell disease is a noninfectious genetic disorder that results in abnormally shaped red blood cells, which can stick together and obstruct the flow of blood through the circulatory system. It is not caused by a pathogen, but rather a genetic mutation. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by Ed Uthman)

Note:



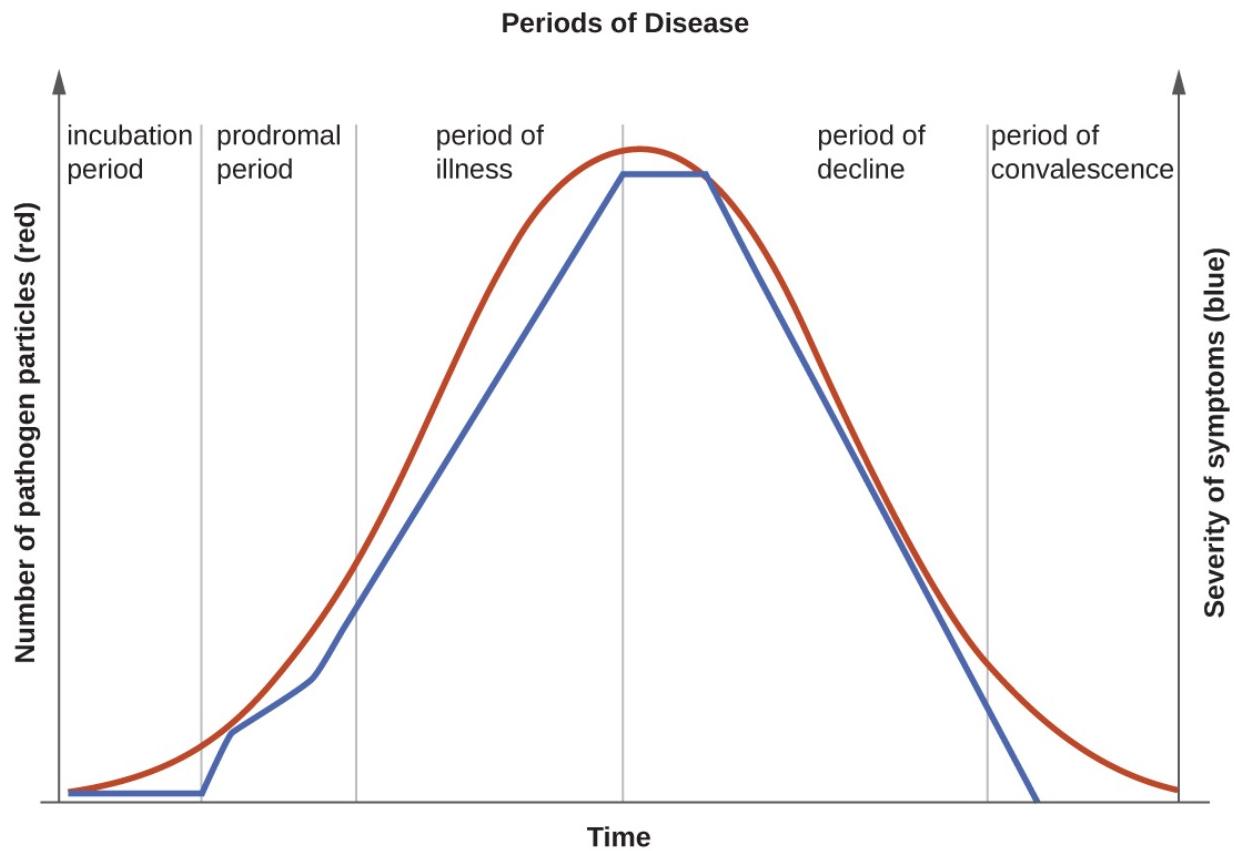
Lists of common infectious diseases can be found at the following [Centers for Disease Control and Prevention](#) (CDC), [World Health Organization](#) (WHO), and [International Classification of Diseases](#) websites.

Note:

- Describe how a disease can be infectious but not contagious.
- Explain the difference between iatrogenic disease and nosocomial disease.

Periods of Disease

The five periods of disease (sometimes referred to as stages or phases) include the incubation, prodromal, illness, decline, and convalescence periods ([\[link\]](#)). The **incubation period** occurs in an acute disease after the initial entry of the pathogen into the host (patient). It is during this time the pathogen begins multiplying in the host. However, there are insufficient numbers of pathogen particles (cells or viruses) present to cause signs and symptoms of disease. Incubation periods can vary from a day or two in acute disease to months or years in chronic disease, depending upon the pathogen. Factors involved in determining the length of the incubation period are diverse, and can include strength of the pathogen, strength of the host immune defenses, site of infection, type of infection, and the size infectious dose received. During this incubation period, the patient is unaware that a disease is beginning to develop.



The progression of an infectious disease can be divided into five periods, which are related to the number of pathogen particles (red) and the severity of signs and symptoms (blue).

The **prodromal period** occurs after the incubation period. During this phase, the pathogen continues to multiply and the host begins to experience general signs and symptoms of illness, which typically result from activation of the immune system, such as fever, pain, soreness, swelling, or inflammation. Usually, such signs and symptoms are too general to indicate a particular disease. Following the prodromal period is the **period of illness**, during which the signs and symptoms of disease are most obvious and severe.

The period of illness is followed by the **period of decline**, during which the number of pathogen particles begins to decrease, and the signs and symptoms of illness begin to decline. However, during the decline period,

patients may become susceptible to developing secondary infections because their immune systems have been weakened by the primary infection. The final period is known as the **period of convalescence**. During this stage, the patient generally returns to normal functions, although some diseases may inflict permanent damage that the body cannot fully repair.

Infectious diseases can be contagious during all five of the periods of disease. Which periods of disease are more likely to be associated with transmissibility of an infection depends upon the disease, the pathogen, and the mechanisms by which the disease develops and progresses. For example, with meningitis (infection of the lining of brain), the periods of infectivity depend on the type of pathogen causing the infection. Patients with bacterial meningitis are contagious during the incubation period for up to a week before the onset of the prodromal period, whereas patients with viral meningitis become contagious when the first signs and symptoms of the prodromal period appear. With many viral diseases associated with rashes (e.g., chickenpox, measles, rubella, roseola), patients are contagious during the incubation period up to a week before the rash develops. In contrast, with many respiratory infections (e.g., colds, influenza, diphtheria, strep throat, and pertussis) the patient becomes contagious with the onset of the prodromal period. Depending upon the pathogen, the disease, and the individual infected, transmission can still occur during the periods of decline, convalescence, and even long after signs and symptoms of the disease disappear. For example, an individual recovering from a diarrheal disease may continue to carry and shed the pathogen in feces for some time, posing a risk of transmission to others through direct contact or indirect contact (e.g., through contaminated objects or food).

Note:

- Name some of the factors that can affect the length of the incubation period of a particular disease.

Acute and Chronic Diseases

The duration of the period of illness can vary greatly, depending on the pathogen, effectiveness of the immune response in the host, and any medical treatment received. For an **acute disease**, pathologic changes occur over a relatively short time (e.g., hours, days, or a few weeks) and involve a rapid onset of disease conditions. For example, influenza (caused by Influenzavirus) is considered an acute disease because the incubation period is approximately 1–2 days. Infected individuals can spread influenza to others for approximately 5 days after becoming ill. After approximately 1 week, individuals enter the period of decline.

For a **chronic disease**, pathologic changes can occur over longer time spans (e.g., months, years, or a lifetime). For example, chronic gastritis (inflammation of the lining of the stomach) is caused by the gram-negative bacterium *Helicobacter pylori*. *H. pylori* is able to colonize the stomach and persist in its highly acidic environment by producing the enzyme urease, which modifies the local acidity, allowing the bacteria to survive indefinitely.[\[footnote\]](#) Consequently, *H. pylori* infections can recur indefinitely unless the infection is cleared using antibiotics.[\[footnote\]](#) Hepatitis B virus can cause a chronic infection in some patients who do not eliminate the virus after the acute illness. A chronic infection with hepatitis B virus is characterized by the continued production of infectious virus for 6 months or longer after the acute infection, as measured by the presence of viral antigen in blood samples.

J.G. Kusters et al. Pathogenesis of *Helicobacter pylori* Infection. *Clinical Microbiology Reviews* 19 no. 3 (2006):449–490.

N.R. Salama et al. “Life in the Human Stomach: Persistence Strategies of the Bacterial Pathogen *Helicobacter pylori*.” *Nature Reviews Microbiology* 11 (2013):385–399.

In **latent diseases**, as opposed to chronic infections, the causal pathogen goes dormant for extended periods of time with no active replication. Examples of diseases that go into a latent state after the acute infection include herpes (herpes simplex viruses [HSV-1 and HSV-2]), chickenpox (varicella-zoster virus [VZV]), and mononucleosis (Epstein-Barr virus [EBV]). HSV-1, HSV-2, and VZV evade the host immune system by

residing in a latent form within cells of the nervous system for long periods of time, but they can reactivate to become active infections during times of stress and immunosuppression. For example, an initial infection by VZV may result in a case of childhood chickenpox, followed by a long period of latency. The virus may reactivate decades later, causing episodes of shingles in adulthood. EBV goes into latency in B cells of the immune system and possibly epithelial cells; it can reactivate years later to produce B-cell lymphoma.

Note:

- Explain the difference between latent disease and chronic disease.

Key Concepts and Summary

- In an **infection**, a microorganism enters a host and begins to multiply. Some infections cause **disease**, which is any deviation from the normal function or structure of the host.
- **Signs** of a disease are objective and are measured. **Symptoms** of a disease are subjective and are reported by the patient.
- Diseases can either be **noninfectious** (due to genetics and environment) or **infectious** (due to pathogens). Some infectious diseases are **communicable** (transmissible between individuals) or **contagious** (easily transmissible between individuals); others are **noncommunicable**, but may be contracted via contact with environmental reservoirs or animals (**zoonoses**)
- **Nosocomial diseases** are contracted in hospital settings, whereas **iatrogenic disease** are the direct result of a medical procedure
- An **acute disease** is short in duration, whereas a **chronic disease** lasts for months or years. **Latent diseases** last for years, but are distinguished from chronic diseases by the lack of active replication during extended dormant periods.

- The periods of disease include the **incubation period**, the **prodromal period**, the **period of illness**, the **period of decline**, and the **period of convalescence**. These periods are marked by changes in the number of infectious agents and the severity of signs and symptoms.

Critical Thinking

Exercise:

Problem:

Two periods of acute disease are the periods of illness and period of decline. (a) In what way are both of these periods similar? (b) In terms of quantity of pathogen, in what way are these periods different? (c) What initiates the period of decline?

Exercise:

Problem:

In July 2015, a report[[footnote](#)] was released indicating the gram-negative bacterium *Pseudomonas aeruginosa* was found on hospital sinks 10 years after the initial outbreak in a neonatal intensive care unit. *P. aeruginosa* usually causes localized ear and eye infections but can cause pneumonia or septicemia in vulnerable individuals like newborn babies. Explain how the current discovery of the presence of this reported *P. aeruginosa* could lead to a recurrence of nosocomial disease.

C. Owens. “*P. aeruginosa* survives in sinks 10 years after hospital outbreak.” 2015. <http://www.healio.com/infectious-disease/nosocomial-infections/news/online/%7B5afba909-56d9-48cc-a9b0-ffe4568161e8%7D/p-aeruginosa-survives-in-sinks-10-years-after-hospital-outbreak>

How Pathogens Cause Disease

LEARNING OBJECTIVES

- Summarize Koch’s postulates and molecular Koch’s postulates, respectively, and explain their significance and limitations
- Explain the concept of pathogenicity (virulence) in terms of infectious and lethal dose
- Distinguish between primary and opportunistic pathogens and identify specific examples of each
- Summarize the stages of pathogenesis
- Explain the roles of portals of entry and exit in the transmission of disease and identify specific examples of these portals

For most infectious diseases, the ability to accurately identify the causative pathogen is a critical step in finding or prescribing effective treatments. Today’s physicians, patients, and researchers owe a sizable debt to the physician Robert Koch (1843–1910), who devised a systematic approach for confirming causative relationships between diseases and specific pathogens.

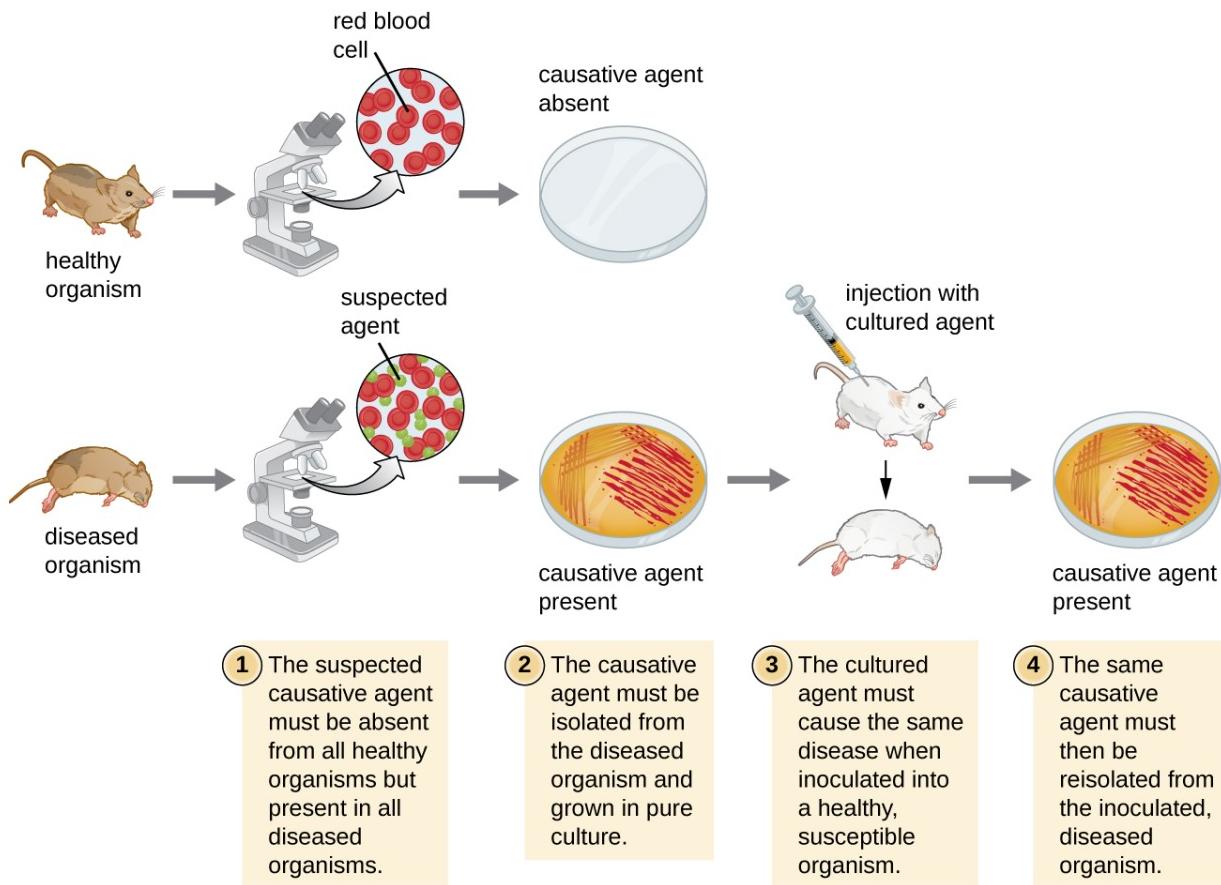
Koch’s Postulates

In 1884, Koch published four postulates ([\[link\]](#)) that summarized his method for determining whether a particular microorganism was the cause of a particular disease. Each of Koch’s postulates represents a criterion that must be met before a disease can be positively linked with a pathogen. In

order to determine whether the criteria are met, tests are performed on laboratory animals and cultures from healthy and diseased animals are compared ([\[link\]](#)).

Koch's Postulates

- (1) The suspected pathogen must be found in every case of disease and not be found in healthy individuals.
- (2) The suspected pathogen can be isolated and grown in pure culture.
- (3) A healthy test subject infected with the suspected pathogen must develop the same signs and symptoms of disease as seen in postulate 1.
- (4) The pathogen must be re-isolated from the new host and must be identical to the pathogen from postulate 2.



The steps for confirming that a pathogen is the cause of a particular disease using Koch's postulates.

In many ways, Koch's postulates are still central to our current understanding of the causes of disease. However, advances in microbiology have revealed some important limitations in Koch's criteria. Koch made several assumptions that we now know are untrue in many cases. The first relates to postulate 1, which assumes that pathogens are only found in diseased, not healthy, individuals. This is not true for many pathogens. For example, *H. pylori*, described earlier in this chapter as a pathogen causing chronic gastritis, is also part of the normal microbiota of the stomach in many healthy humans who never develop gastritis. It is estimated that upwards of 50% of the human population acquires *H. pylori* early in life, with most maintaining it as part of the normal microbiota for the rest of their life without ever developing disease.

Koch's second faulty assumption was that all healthy test subjects are equally susceptible to disease. We now know that individuals are not equally susceptible to disease. Individuals are unique in terms of their microbiota and the state of their immune system at any given time. The makeup of the resident microbiota can influence an individual's susceptibility to an infection. Members of the normal microbiota play an important role in immunity by inhibiting the growth of transient pathogens. In some cases, the microbiota may prevent a pathogen from establishing an infection; in others, it may not prevent an infection altogether but may influence the severity or type of signs and symptoms. As a result, two individuals with the same disease may not always present with the same signs and symptoms. In addition, some individuals have stronger immune systems than others. Individuals with immune systems weakened by age or an unrelated illness are much more susceptible to certain infections than individuals with strong immune systems.

Koch also assumed that all pathogens are microorganisms that can be grown in pure culture (postulate 2) and that animals could serve as reliable models for human disease. However, we now know that not all pathogens can be grown in pure culture, and many human diseases cannot be reliably replicated in animal hosts. Viruses and certain bacteria, including *Rickettsia* and *Chlamydia*, are obligate intracellular pathogens that can grow only when inside a host cell. If a microbe cannot be cultured, a researcher cannot move past postulate 2. Likewise, without a suitable nonhuman host, a researcher cannot evaluate postulate 2 without deliberately infecting humans, which presents obvious ethical concerns. AIDS is an example of such a disease because the human immunodeficiency virus (HIV) only causes disease in humans.

Note:

- Briefly summarize the limitations of Koch's postulates.

Molecular Koch's Postulates

In 1988, Stanley Falkow (1934–) proposed a revised form of Koch's postulates known as molecular Koch's postulates. These are listed in the left column of [link]. The premise for molecular Koch's postulates is not in the ability to isolate a particular pathogen but rather to identify a gene that may cause the organism to be pathogenic.

Falkow's modifications to Koch's original postulates explain not only infections caused by intracellular pathogens but also the existence of pathogenic strains of organisms that are usually nonpathogenic. For example, the predominant form of the bacterium *Escherichia coli* is a member of the normal microbiota of the human intestine and is generally considered harmless. However, there are pathogenic strains of *E. coli* such as enterotoxigenic *E. coli* (ETEC) and enterohemorrhagic *E. coli* (O157:H7) (EHEC). We now know ETEC and EHEC exist because of the acquisition of new genes by the once-harmless *E. coli*, which, in the form of these pathogenic strains, is now capable of producing toxins and causing illness. The pathogenic forms resulted from minor genetic changes. The right-side column of [link] illustrates how molecular Koch's postulates can be applied to identify EHEC as a pathogenic bacterium.

Molecular Koch's Postulates Applied to EHEC

Molecular Koch's Postulates	Application to EHEC
(1) The phenotype (sign or symptom of disease) should be associated only with pathogenic strains of a species.	EHEC causes intestinal inflammation and diarrhea, whereas nonpathogenic strains of <i>E. coli</i> do not.

Molecular Koch's Postulates Applied to EHEC	
Molecular Koch's Postulates	Application to EHEC
(2) Inactivation of the suspected gene(s) associated with pathogenicity should result in a measurable loss of pathogenicity.	One of the genes in EHEC encodes for Shiga toxin, a bacterial toxin (poison) that inhibits protein synthesis. Inactivating this gene reduces the bacteria's ability to cause disease.
(3) Reversion of the inactive gene should restore the disease phenotype.	By adding the gene that encodes the toxin back into the genome (e.g., with a phage or plasmid), EHEC's ability to cause disease is restored.

As with Koch's original postulates, the molecular Koch's postulates have limitations. For example, genetic manipulation of some pathogens is not possible using current methods of molecular genetics. In a similar vein, some diseases do not have suitable animal models, which limits the utility of both the original and molecular postulates.

Note:

- Explain the differences between Koch's original postulates and the molecular Koch's postulates.

Pathogenicity and Virulence

The ability of a microbial agent to cause disease is called **pathogenicity**, and the degree to which an organism is pathogenic is called **virulence**.

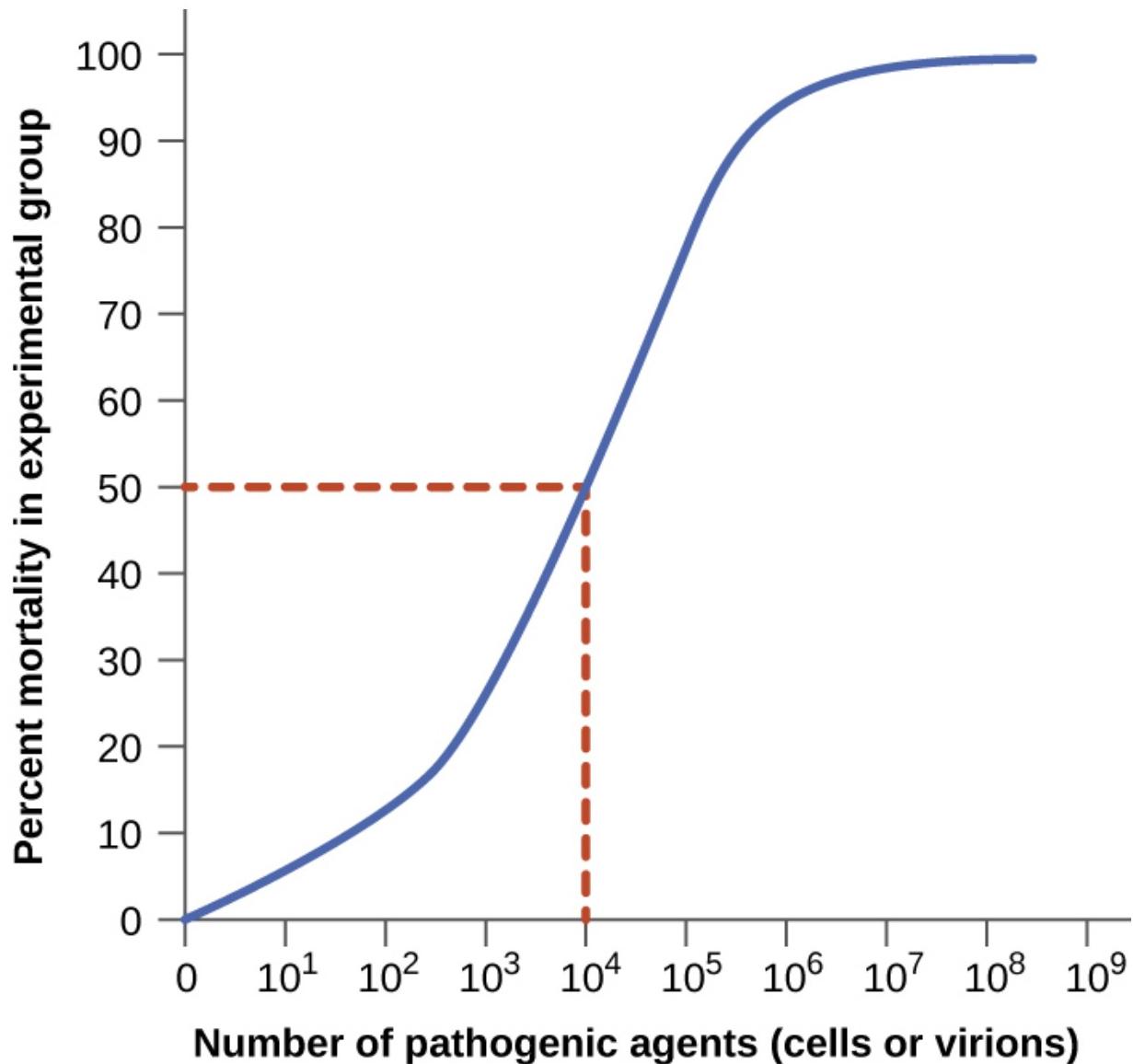
Virulence is a continuum. On one end of the spectrum are organisms that are avirulent (not harmful) and on the other are organisms that are highly virulent. Highly virulent pathogens will almost always lead to a disease state when introduced to the body, and some may even cause multi-organ and body system failure in healthy individuals. Less virulent pathogens may cause an initial infection, but may not always cause severe illness.

Pathogens with low virulence would more likely result in mild signs and symptoms of disease, such as low-grade fever, headache, or muscle aches. Some individuals might even be asymptomatic.

An example of a highly virulent microorganism is *Bacillus anthracis*, the pathogen responsible for anthrax. *B. anthracis* can produce different forms of disease, depending on the route of transmission (e.g., cutaneous injection, inhalation, ingestion). The most serious form of anthrax is inhalation anthrax. After *B. anthracis* spores are inhaled, they germinate. An active infection develops and the bacteria release potent toxins that cause edema (fluid buildup in tissues), hypoxia (a condition preventing oxygen from reaching tissues), and necrosis (cell death and inflammation). Signs and symptoms of inhalation anthrax include high fever, difficulty breathing, vomiting and coughing up blood, and severe chest pains suggestive of a heart attack. With inhalation anthrax, the toxins and bacteria enter the bloodstream, which can lead to multi-organ failure and death of the patient. If a gene (or genes) involved in pathogenesis is inactivated, the bacteria become less virulent or nonpathogenic.

Virulence of a pathogen can be quantified using controlled experiments with laboratory animals. Two important indicators of virulence are the **median infectious dose (ID_{50})** and the **median lethal dose (LD_{50})**, both of which are typically determined experimentally using animal models. The ID_{50} is the number of pathogen cells or virions required to cause active infection in 50% of inoculated animals. The LD_{50} is the number of pathogenic cells, virions, or amount of toxin required to kill 50% of infected animals. To calculate these values, each group of animals is inoculated with one of a range of known numbers of pathogen cells or virions. In graphs like the one shown in [\[link\]](#), the percentage of animals that have been infected (for ID_{50}) or killed (for LD_{50}) is plotted against the concentration of pathogen inoculated. [\[link\]](#) represents data graphed from a

hypothetical experiment measuring the LD₅₀ of a pathogen. Interpretation of the data from this graph indicates that the LD₅₀ of the pathogen for the test animals is 10⁴ pathogen cells or virions (depending upon the pathogen studied).



A graph like this is used to determine LD₅₀ by plotting pathogen concentration against the percent of infected test animals that have died. In this example, the LD₅₀ = 10⁴ pathogenic particles.

[\[link\]](#) lists selected foodborne pathogens and their ID₅₀ values in humans (as determined from epidemiologic data and studies on human volunteers). Keep in mind that these are *median* values. The actual infective dose for an individual can vary widely, depending on factors such as route of entry; the age, health, and immune status of the host; and environmental and pathogen-specific factors such as susceptibility to the acidic pH of the stomach. It is also important to note that a pathogen's infective dose does not necessarily correlate with disease severity. For example, just a single cell of *Salmonella enterica* serotype Typhimurium can result in an active infection. The resultant disease, *Salmonella* gastroenteritis or salmonellosis, can cause nausea, vomiting, and diarrhea, but has a mortality rate of less than 1% in healthy adults. In contrast, *S. enterica* serotype Typhi has a much higher ID₅₀, typically requiring as many as 1,000 cells to produce infection. However, this serotype causes typhoid fever, a much more systemic and severe disease that has a mortality rate as high as 10% in untreated individuals.

ID₅₀ for Selected Foodborne Diseases[\[footnote\]](#)

Food and Drug Administration. “Bad Bug Book, Foodborne Pathogenic Microorganisms and Natural Toxins.” 2nd ed. Silver Spring, MD: US Food and Drug Administration; 2012.

Pathogen	ID ₅₀
Viruses	
Hepatitis A virus	10–100
Norovirus	1–10
Rotavirus	10–100

ID₅₀ for Selected Foodborne Diseases[\[footnote\]](#)

Food and Drug Administration. “Bad Bug Book, Foodborne Pathogenic Microorganisms and Natural Toxins.” 2nd ed. Silver Spring, MD: US Food and Drug Administration; 2012.

Pathogen	ID₅₀
Bacteria	
<i>Escherichia coli</i> , enterohemorrhagic (EHEC, serotype O157)	10–100
<i>E. coli</i> , enteroinvasive (EIEC)	200–5,000
<i>E. coli</i> , enteropathogenic (EPEC)	10,000,000–10,000,000,000
<i>E. coli</i> , enterotoxigenic (ETEC)	10,000,000–10,000,000,000
<i>Salmonella enterica</i> serovar Typhi	<1,000
<i>S. enterica</i> serovar Typhimurium	≥1
<i>Shigella dysenteriae</i>	10–200
<i>Vibrio cholerae</i> (serotypes O139, O1)	1,000,000
<i>V. parahemolyticus</i>	100,000,000
Protozoa	
<i>Giardia lamblia</i>	1
<i>Cryptosporidium parvum</i>	10–100

Note:

- What is the difference between a pathogen's infective dose and lethal dose?
- Which is more closely related to the severity of a disease?

Primary Pathogens versus Opportunistic Pathogens

Pathogens can be classified as either primary pathogens or opportunistic pathogens. A **primary pathogen** can cause disease in a host regardless of the host's resident microbiota or immune system. An **opportunistic pathogen**, by contrast, can only cause disease in situations that compromise the host's defenses, such as the body's protective barriers, immune system, or normal microbiota. Individuals susceptible to opportunistic infections include the very young, the elderly, women who are pregnant, patients undergoing chemotherapy, people with immunodeficiencies (such as acquired immunodeficiency syndrome [AIDS]), patients who are recovering from surgery, and those who have had a breach of protective barriers (such as a severe wound or burn).

An example of a primary pathogen is enterohemorrhagic *E. coli* (EHEC), which produces a virulence factor known as Shiga toxin. This toxin inhibits protein synthesis, leading to severe and bloody diarrhea, inflammation, and renal failure, even in patients with healthy immune systems. *Staphylococcus epidermidis*, on the other hand, is an opportunistic pathogen that is among the most frequent causes of nosocomial disease.[\[footnote\]](#) *S. epidermidis* is a member of the normal microbiota of the skin, where it is generally avirulent. However, in hospitals, it can also grow in biofilms that form on catheters, implants, or other devices that are inserted into the body during surgical procedures. Once inside the body, *S. epidermidis* can cause serious infections such as endocarditis, and it produces virulence factors that promote the persistence of such infections.

M. Otto. “*Staphylococcus epidermidis*--The ‘Accidental’ Pathogen.” *Nature Reviews Microbiology* 7 no. 8 (2009):555–567.

Other members of the normal microbiota can also cause opportunistic infections under certain conditions. This often occurs when microbes that reside harmlessly in one body location end up in a different body system, where they cause disease. For example, *E. coli* normally found in the large intestine can cause a urinary tract infection if it enters the bladder. This is the leading cause of urinary tract infections among women.

Members of the normal microbiota may also cause disease when a shift in the environment of the body leads to overgrowth of a particular microorganism. For example, the yeast *Candida* is part of the normal microbiota of the skin, mouth, intestine, and vagina, but its population is kept in check by other organisms of the microbiota. If an individual is taking antibacterial medications, however, bacteria that would normally inhibit the growth of *Candida* can be killed off, leading to a sudden growth in the population of *Candida*, which is not affected by antibacterial medications because it is a fungus. An overgrowth of *Candida* can manifest as oral thrush (growth on mouth, throat, and tongue), a vaginal yeast infection, or cutaneous candidiasis. Other scenarios can also provide opportunities for *Candida* infections. Untreated diabetes can result in a high concentration of glucose in the saliva, which provides an optimal environment for the growth of *Candida*, resulting in thrush. Immunodeficiencies such as those seen in patients with HIV, AIDS, and cancer also lead to higher incidence of thrush. Vaginal yeast infections can result from decreases in estrogen levels during the menstruation or menopause. The amount of glycogen available to lactobacilli in the vagina is controlled by levels of estrogen; when estrogen levels are low, lactobacilli produce less lactic acid. The resultant increase in vaginal pH allows overgrowth of *Candida* in the vagina.

Note:

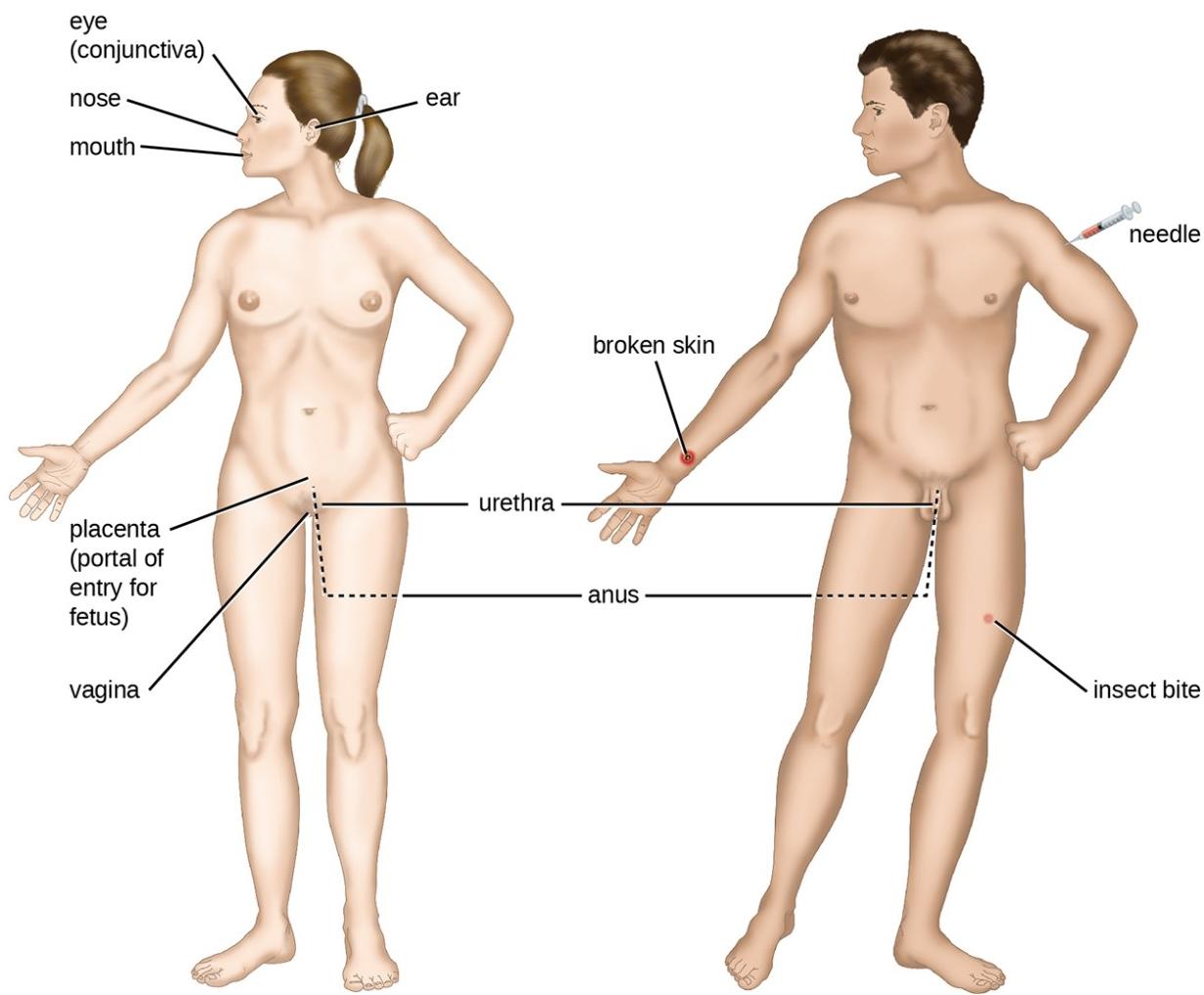
- Explain the difference between a primary pathogen and an opportunistic pathogen.
- Describe some conditions under which an opportunistic infection can occur.

Stages of Pathogenesis

To cause disease, a pathogen must successfully achieve four steps or stages of pathogenesis: exposure (contact), adhesion (colonization), invasion, and infection. The pathogen must be able to gain entry to the host, travel to the location where it can establish an infection, evade or overcome the host's immune response, and cause damage (i.e., disease) to the host. In many cases, the cycle is completed when the pathogen exits the host and is transmitted to a new host.

Exposure

An encounter with a potential pathogen is known as **exposure** or **contact**. The food we eat and the objects we handle are all ways that we can come into contact with potential pathogens. Yet, not all contacts result in infection and disease. For a pathogen to cause disease, it needs to be able to gain access into host tissue. An anatomic site through which pathogens can pass into host tissue is called a **portal of entry**. These are locations where the host cells are in direct contact with the external environment. Major portals of entry are identified in [\[link\]](#) and include the skin, mucous membranes, and parenteral routes.



Shown are different portals of entry where pathogens can gain access into the body. With the exception of the placenta, many of these locations are directly exposed to the external environment.

Mucosal surfaces are the most important portals of entry for microbes; these include the mucous membranes of the respiratory tract, the gastrointestinal tract, and the genitourinary tract. Although most mucosal surfaces are in the interior of the body, some are contiguous with the external skin at various body openings, including the eyes, nose, mouth, urethra, and anus.

Most pathogens are suited to a particular portal of entry. A pathogen's portal specificity is determined by the organism's environmental adaptions

and by the enzymes and toxins they secrete. The respiratory and gastrointestinal tracts are particularly vulnerable portals of entry because particles that include microorganisms are constantly inhaled or ingested, respectively.

Pathogens can also enter through a breach in the protective barriers of the skin and mucous membranes. Pathogens that enter the body in this way are said to enter by the **parenteral route**. For example, the skin is a good natural barrier to pathogens, but breaks in the skin (e.g., wounds, insect bites, animal bites, needle pricks) can provide a parenteral portal of entry for microorganisms.

In pregnant women, the placenta normally prevents microorganisms from passing from the mother to the fetus. However, a few pathogens are capable of crossing the blood-placental barrier. The gram-positive bacterium *Listeria monocytogenes*, which causes the foodborne disease listeriosis, is one example that poses a serious risk to the fetus and can sometimes lead to spontaneous abortion. Other pathogens that can pass the placental barrier to infect the fetus are known collectively by the acronym TORCH ([\[link\]](#)).

Transmission of infectious diseases from mother to baby is also a concern at the time of birth when the baby passes through the birth canal. Babies whose mothers have active chlamydia or gonorrhea infections may be exposed to the causative pathogens in the vagina, which can result in eye infections that lead to blindness. To prevent this, it is standard practice to administer antibiotic drops to infants' eyes shortly after birth.

Pathogens Capable of Crossing the Placental Barrier (TORCH Infections)

	Disease	Pathogen
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Pathogens Capable of Crossing the Placental Barrier (TORCH Infections)

	Disease	Pathogen
T	Toxoplasmosis	<i>Toxoplasma gondii</i> (protozoan)
O <u>[footnote]</u> The O in TORCH stands for “other.”	Syphilis Chickenpox Hepatitis B HIV Fifth disease (erythema infectiosum)	<i>Treponema pallidum</i> (bacterium) Varicella-zoster virus (human herpesvirus 3) Hepatitis B virus (hepatnavirus) Retrovirus Parvovirus B19
R	Rubella (German measles)	Togavirus
C	Cytomegalovirus	Human herpesvirus 5
H	Herpes	Herpes simplex viruses (HSV) 1 and 2

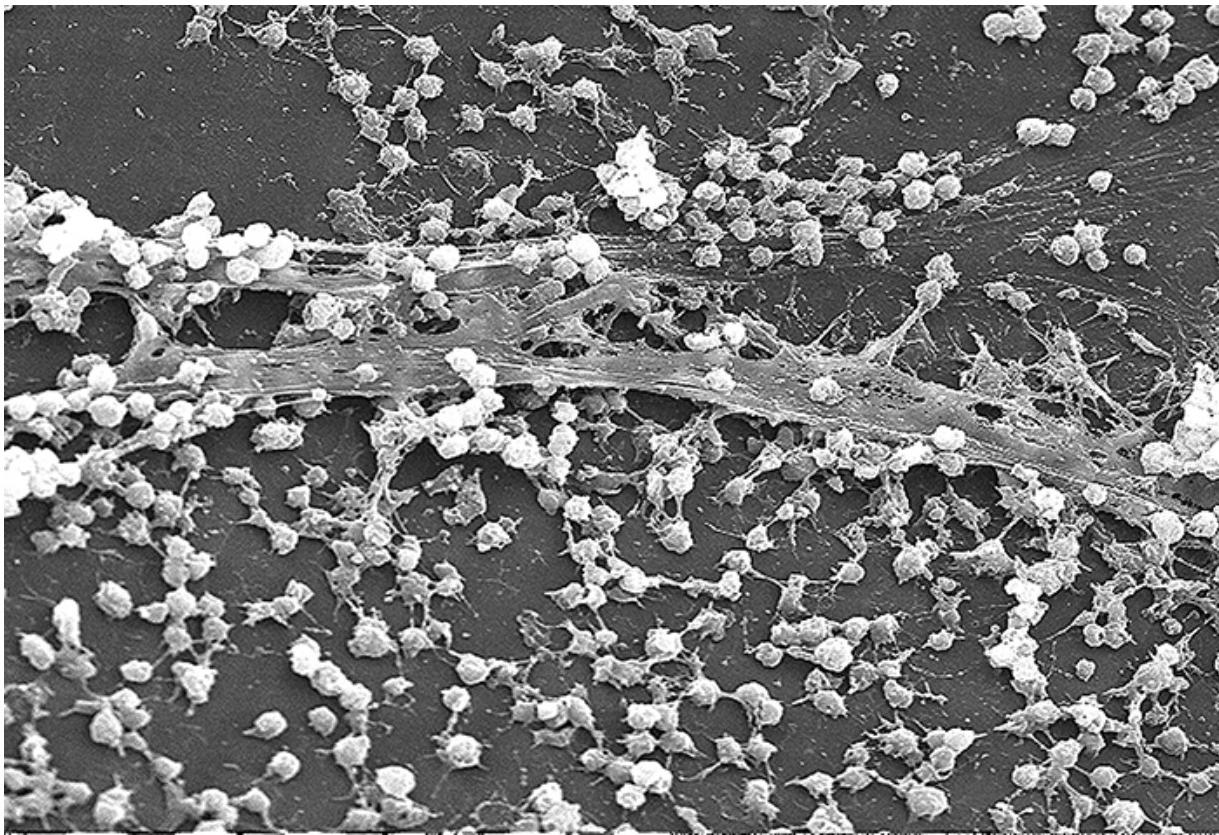
Adhesion

Following the initial exposure, the pathogen adheres at the portal of entry. The term **adhesion** refers to the capability of pathogenic microbes to attach to the cells of the body using adhesion factors, and different pathogens use various mechanisms to adhere to the cells of host tissues.

Molecules (either proteins or carbohydrates) called **adhesins** are found on the surface of certain pathogens and bind to specific receptors (glycoproteins) on host cells. Adhesins are present on the fimbriae and

flagella of bacteria, the cilia of protozoa, and the capsids or membranes of viruses. Protozoans can also use hooks and barbs for adhesion; spike proteins on viruses also enhance viral adhesion. The production of glycocalyces (slime layers and capsules) ([\[link\]](#)), with their high sugar and protein content, can also allow certain bacterial pathogens to attach to cells.

Biofilm growth can also act as an adhesion factor. A biofilm is a community of bacteria that produce a glycocalyx, known as extrapolymeric substance (EPS), that allows the biofilm to attach to a surface. Persistent *Pseudomonas aeruginosa* infections are common in patients suffering from cystic fibrosis, burn wounds, and middle-ear infections (otitis media) because *P. aeruginosa* produces a biofilm. The EPS allows the bacteria to adhere to the host cells and makes it harder for the host to physically remove the pathogen. The EPS not only allows for attachment but provides protection against the immune system and antibiotic treatments, preventing antibiotics from reaching the bacterial cells within the biofilm. In addition, not all bacteria in a biofilm are rapidly growing; some are in stationary phase. Since antibiotics are most effective against rapidly growing bacteria, portions of bacteria in a biofilm are protected against antibiotics.[\[footnote\]](#)
D. Davies. “Understanding Biofilm Resistance to Antibacterial Agents.” *Nature Reviews Drug Discovery* 2 (2003):114–122.

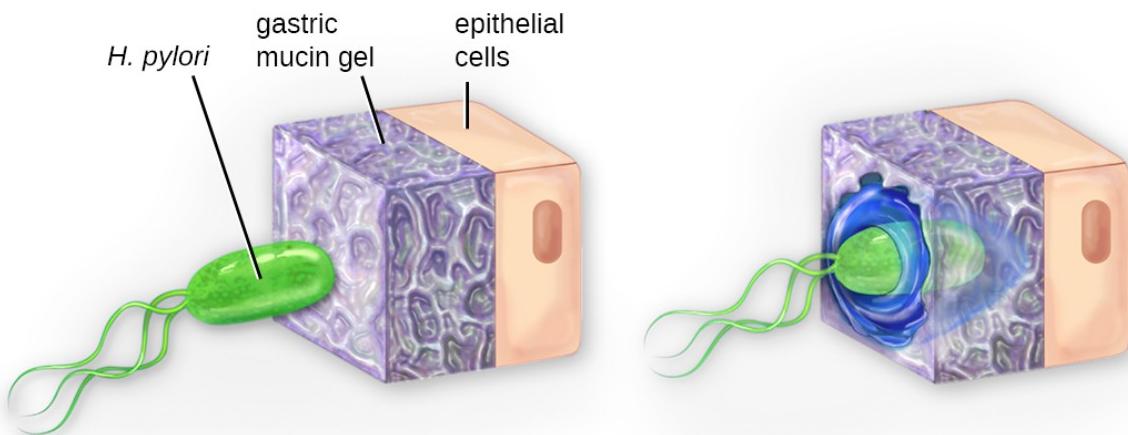


Glycocalyx produced by bacteria in a biofilm allows the cells to adhere to host tissues and to medical devices such as the catheter surface shown here. (credit: modification of work by Centers for Disease Control and Prevention)

Invasion

Once adhesion is successful, **invasion** can proceed. Invasion involves the dissemination of a pathogen throughout local tissues or the body. Pathogens may produce exoenzymes or toxins, which serve as virulence factors that allow them to colonize and damage host tissues as they spread deeper into the body. Pathogens may also produce virulence factors that protect them against immune system defenses. A pathogen's specific virulence factors determine the degree of tissue damage that occurs. [\[link\]](#) shows the

invasion of *H. pylori* into the tissues of the stomach, causing damage as it progresses.



Contact with stomach acid keeps the mucin lining the epithelial cell layer in a spongy gel-like state. This consistency is impermeable to the bacterium *H. pylori*.

The bacterium releases urease, which neutralizes the stomach acid. This causes the mucin to liquefy, and the bacterium can swim right through it.

H. pylori is able to invade the lining of the stomach by producing virulence factors that enable it pass through the mucin layer covering epithelial cells. (credit: modification of work by Zina Deretsky, National Science Foundation)

Intracellular pathogens achieve invasion by entering the host's cells and reproducing. Some are obligate intracellular pathogens (meaning they can only reproduce inside of host cells) and others are facultative intracellular pathogens (meaning they can reproduce either inside or outside of host cells). By entering the host cells, intracellular pathogens are able to evade some mechanisms of the immune system while also exploiting the nutrients in the host cell.

Entry to a cell can occur by endocytosis. For most kinds of host cells, pathogens use one of two different mechanisms for endocytosis and entry. One mechanism relies on effector proteins secreted by the pathogen; these

effector proteins trigger entry into the host cell. This is the method that *Salmonella* and *Shigella* use when invading intestinal epithelial cells. When these pathogens come in contact with epithelial cells in the intestine, they secrete effector molecules that cause protrusions of membrane ruffles that bring the bacterial cell in. This process is called membrane ruffling. The second mechanism relies on surface proteins expressed on the pathogen that bind to receptors on the host cell, resulting in entry. For example, *Yersinia pseudotuberculosis* produces a surface protein known as invasin that binds to beta-1 integrins expressed on the surface of host cells.

Some host cells, such as white blood cells and other phagocytes of the immune system, actively endocytose pathogens in a process called phagocytosis. Although phagocytosis allows the pathogen to gain entry to the host cell, in most cases, the host cell kills and degrades the pathogen by using digestive enzymes. Normally, when a pathogen is ingested by a phagocyte, it is enclosed within a phagosome in the cytoplasm; the phagosome fuses with a lysosome to form a phagolysosome, where digestive enzymes kill the pathogen (see [Pathogen Recognition and Phagocytosis](#)). However, some intracellular pathogens have the ability to survive and multiply within phagocytes. Examples include *Listeria monocytogenes* and *Shigella*; these bacteria produce proteins that lyse the phagosome before it fuses with the lysosome, allowing the bacteria to escape into the phagocyte's cytoplasm where they can multiply. Bacteria such as *Mycobacterium tuberculosis*, *Legionella pneumophila*, and *Salmonella* species use a slightly different mechanism to evade being digested by the phagocyte. These bacteria prevent the fusion of the phagosome with the lysosome, thus remaining alive and dividing within the phagosome.

Infection

Following invasion, successful multiplication of the pathogen leads to infection. Infections can be described as local, focal, or systemic, depending on the extent of the infection. A **local infection** is confined to a small area of the body, typically near the portal of entry. For example, a hair follicle infected by *Staphylococcus aureus* infection may result in a boil around the

site of infection, but the bacterium is largely contained to this small location. Other examples of local infections that involve more extensive tissue involvement include urinary tract infections confined to the bladder or pneumonia confined to the lungs.

In a **focal infection**, a localized pathogen, or the toxins it produces, can spread to a secondary location. For example, a dental hygienist nicking the gum with a sharp tool can lead to a local infection in the gum by *Streptococcus* bacteria of the normal oral microbiota. These *Streptococcus* spp. may then gain access to the bloodstream and make their way to other locations in the body, resulting in a secondary infection.

When an infection becomes disseminated throughout the body, we call it a **systemic infection**. For example, infection by the varicella-zoster virus typically gains entry through a mucous membrane of the upper respiratory system. It then spreads throughout the body, resulting in the classic red skin lesions associated with chickenpox. Since these lesions are not sites of initial infection, they are signs of a systemic infection.

Sometimes a **primary infection**, the initial infection caused by one pathogen, can lead to a **secondary infection** by another pathogen. For example, the immune system of a patient with a primary infection by HIV becomes compromised, making the patient more susceptible to secondary diseases like oral thrush and others caused by opportunistic pathogens. Similarly, a primary infection by Influenzavirus damages and decreases the defense mechanisms of the lungs, making patients more susceptible to a secondary pneumonia by a bacterial pathogen like *Haemophilus influenzae* or *Streptococcus pneumoniae*. Some secondary infections can even develop as a result of treatment for a primary infection. Antibiotic therapy targeting the primary pathogen can cause collateral damage to the normal microbiota, creating an opening for opportunistic pathogens (see [Case in Point: A Secondary Yeast Infection](#)).

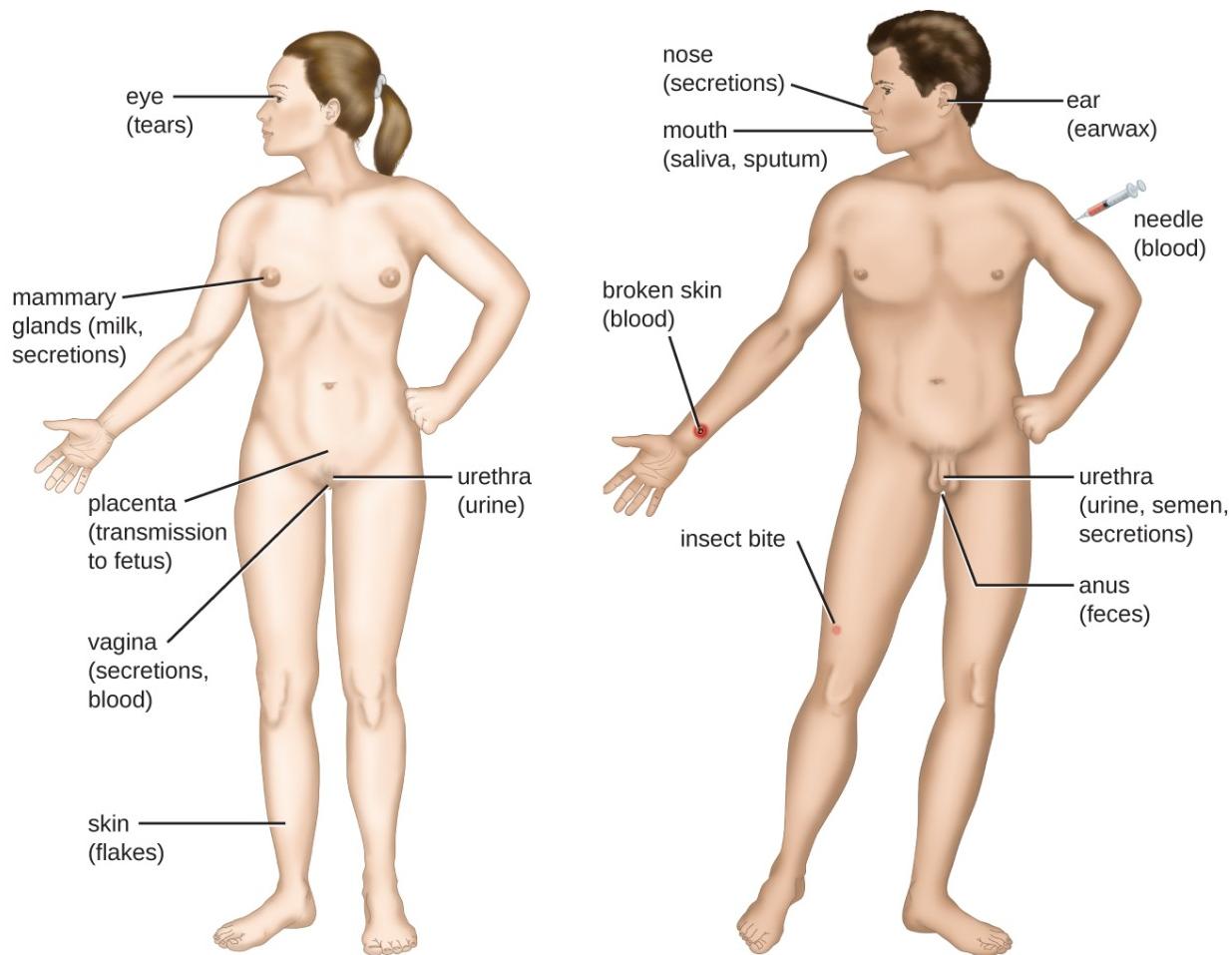
Note:

- List three conditions that could lead to a secondary infection.

Transmission of Disease

For a pathogen to persist, it must put itself in a position to be transmitted to a new host, leaving the infected host through a **portal of exit** ([\[link\]](#)). As with portals of entry, many pathogens are adapted to use a particular portal of exit. Similar to portals of entry, the most common portals of exit include the skin and the respiratory, urogenital, and gastrointestinal tracts.

Coughing and sneezing can expel pathogens from the respiratory tract. A single sneeze can send thousands of virus particles into the air. Secretions and excretions can transport pathogens out of other portals of exit. Feces, urine, semen, vaginal secretions, tears, sweat, and shed skin cells can all serve as vehicles for a pathogen to leave the body. Pathogens that rely on insect vectors for transmission exit the body in the blood extracted by a biting insect. Similarly, some pathogens exit the body in blood extracted by needles.



Pathogens leave the body of an infected host through various portals of exit to infect new hosts.

Key Concepts and Summary

- **Koch's postulates** are used to determine whether a particular microorganism is a pathogen. **Molecular Koch's postulates** are used to determine what genes contribute to a pathogen's ability to cause disease.
- **Virulence**, the degree to which a pathogen can cause disease, can be quantified by calculating either the **ID₅₀** or **LD₅₀** of a pathogen on a given population.

- **Primary pathogens** are capable of causing pathological changes associated with disease in a healthy individual, whereas **opportunistic pathogens** can only cause disease when the individual is compromised by a break in protective barriers or immunosuppression.
- Infections and disease can be caused by pathogens in the environment or microbes in an individual's **resident microbiota**.
- Infections can be classified as **local**, **focal**, or **systemic** depending on the extent to which the pathogen spreads in the body.
- A **secondary infection** can sometimes occur after the host's defenses or normal microbiota are compromised by a **primary infection** or antibiotic treatment.
- Pathogens enter the body through **portals of entry** and leave through **portals of exit**. The stages of pathogenesis include **exposure**, **adhesion**, **invasion**, **infection**, and **transmission**.

Critical Thinking

Exercise:

Problem:

Diseases that involve biofilm-producing bacteria are of serious concern. They are not as easily treated compared with those involving free-floating (or planktonic) bacteria. Explain three reasons why biofilm formers are more pathogenic.

Exercise:

Problem:

A microbiologist has identified a new gram-negative pathogen that causes liver disease in rats. She suspects that the bacterium's fimbriae are a virulence factor. Describe how molecular Koch's postulates could be used to test this hypothesis.

Exercise:

Problem:

Acupuncture is a form of alternative medicine that is used for pain relief. Explain how acupuncture could facilitate exposure to pathogens.



Virulence Factors of Bacterial and Viral Pathogens

LEARNING OBJECTIVES

- Explain how virulence factors contribute to signs and symptoms of infectious disease
- Differentiate between endotoxins and exotoxins
- Describe and differentiate between various types of exotoxins
- Describe the mechanisms viruses use for adhesion and antigenic variation

In the previous section, we explained that some pathogens are more virulent than others. This is due to the unique **virulence factors** produced by individual pathogens, which determine the extent and severity of disease they may cause. A pathogen's virulence factors are encoded by genes that can be identified using molecular Koch's postulates. When genes encoding virulence factors are inactivated, virulence in the pathogen is diminished. In this section, we examine various types and specific examples of virulence factors and how they contribute to each step of pathogenesis.

Virulence Factors for Adhesion

As discussed in the previous section, the first two steps in pathogenesis are exposure and adhesion. Recall that an adhesin is a protein or glycoprotein found on the surface of a pathogen that attaches to receptors on the host cell. Adhesins are found on bacterial, viral, fungal, and protozoan pathogens. One example of a bacterial adhesin is type 1 fimbrial adhesin, a molecule found on the tips of fimbriae of enterotoxigenic *E. coli* (ETEC). Recall that fimbriae are hairlike protein bristles on the cell surface. Type 1 fimbrial adhesin allows the fimbriae of ETEC cells to attach to the mannose glycans expressed on intestinal epithelial cells. [\[link\]](#) lists common adhesins found in some of the pathogens we have discussed or will be seeing later in this chapter.

Some Bacterial Adhesins and Their Host Attachment Sites

Pathogen	Disease	Adhesin	Attachment Site
<i>Streptococcus pyogenes</i>	Strep throat	Protein F	Respiratory epithelial cells
<i>Streptococcus mutans</i>	Dental caries	Adhesin P1	Teeth
<i>Neisseria gonorrhoeae</i>	Gonorrhea	Type IV pili	Urethral epithelial cells
Enterotoxigenic <i>E. coli</i> (ETEC)	Traveler's diarrhea	Type 1 fimbriae	Intestinal epithelial cells
<i>Vibrio cholerae</i>	Cholera	N-methylphenylalanine pili	Intestinal epithelial cells

Bacterial Exoenzymes and Toxins as Virulence Factors

After exposure and adhesion, the next step in pathogenesis is invasion, which can involve enzymes and toxins. Many pathogens achieve invasion by entering the bloodstream, an effective means of dissemination because blood vessels pass close to every cell in the body. The downside of this mechanism of dispersal is that the blood also includes numerous elements of the immune system. Various terms ending in –emia are used to describe the presence of pathogens in the bloodstream. The presence of bacteria in blood is called **bacteremia**. Bacteremia involving pyogens (pus-forming bacteria) is called pyemia. When viruses are found in the blood, it is called **viremia**. The term **toxemia** describes the condition when toxins are found in the blood. If bacteria are both present and multiplying in the blood, this condition is called **septicemia**.

Patients with septicemia are described as **septic**, which can lead to **shock**, a life-threatening decrease in blood pressure (systolic pressure <90 mm Hg) that prevents

cells and organs from receiving enough oxygen and nutrients. Some bacteria can cause shock through the release of toxins (virulence factors that can cause tissue damage) and lead to low blood pressure. Gram-negative bacteria are engulfed by immune system phagocytes, which then release tumor necrosis factor, a molecule involved in inflammation and fever. Tumor necrosis factor binds to blood capillaries to increase their permeability, allowing fluids to pass out of blood vessels and into tissues, causing swelling, or edema ([\[link\]](#)). With high concentrations of tumor necrosis factor, the inflammatory reaction is severe and enough fluid is lost from the circulatory system that blood pressure decreases to dangerously low levels. This can have dire consequences because the heart, lungs, and kidneys rely on normal blood pressure for proper function; thus, multi-organ failure, shock, and death can occur.



This patient has edema in the tissue of the right hand. Such swelling can occur when bacteria cause the release of pro-inflammatory molecules from immune

cells and these molecules cause an increased permeability of blood vessels, allowing fluid to escape the bloodstream and enter tissue.

Exoenzymes

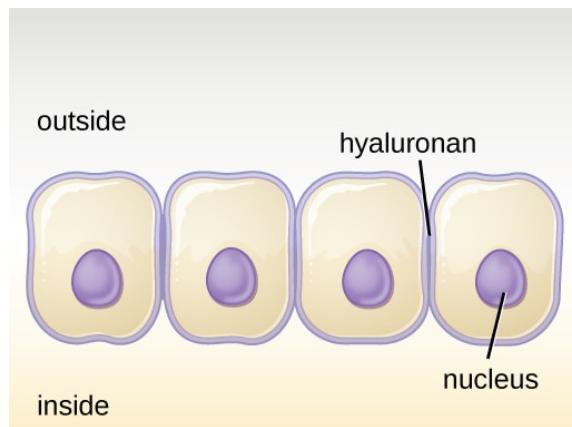
Some pathogens produce extracellular enzymes, or **exoenzymes**, that enable them to invade host cells and deeper tissues. Exoenzymes have a wide variety of targets. Some general classes of exoenzymes and associated pathogens are listed in [[link](#)]. Each of these exoenzymes functions in the context of a particular tissue structure to facilitate invasion or support its own growth and defend against the immune system. For example, **hyaluronidase S**, an enzyme produced by pathogens like *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Clostridium perfringens*, degrades the glycoside hyaluronan (hyaluronic acid), which acts as an intercellular cement between adjacent cells in connective tissue ([\[link\]](#)). This allows the pathogen to pass through the tissue layers at the portal of entry and disseminate elsewhere in the body ([\[link\]](#)).

Some Classes of Exoenzymes and Their Targets

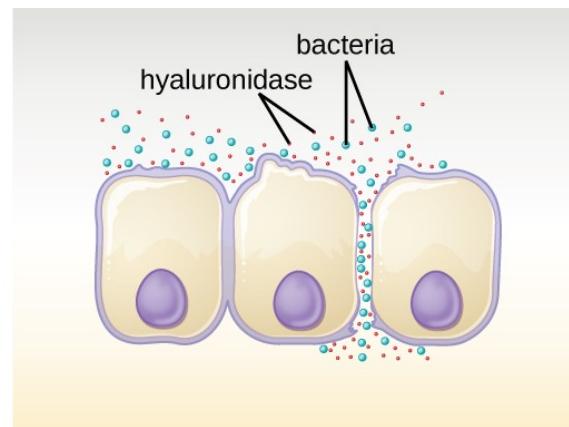
Class	Example	Function
Glycohydrolases	Hyaluronidase S in <i>Staphylococcus aureus</i>	Degrades hyaluronic acid that cements cells together to promote spreading through tissues
Nucleases	DNAse produced by <i>S. aureus</i>	Degrades DNA released by dying cells (bacteria and host cells) that can trap the bacteria, thus promoting spread

Some Classes of Exoenzymes and Their Targets

Class	Example	Function
Phospholipases	Phospholipase C of <i>Bacillus anthracis</i>	Degradates phospholipid bilayer of host cells, causing cellular lysis, and degrade membrane of phagosomes to enable escape into the cytoplasm
Proteases	Collagenase in <i>Clostridium perfringens</i>	Degradates collagen in connective tissue to promote spread



(a)



(b)

(a) Hyaluronan is a polymer found in the layers of epidermis that connect adjacent cells. (b) Hyaluronidase produced by bacteria degrades this adhesive polymer in the extracellular matrix, allowing passage between cells that would otherwise be blocked.

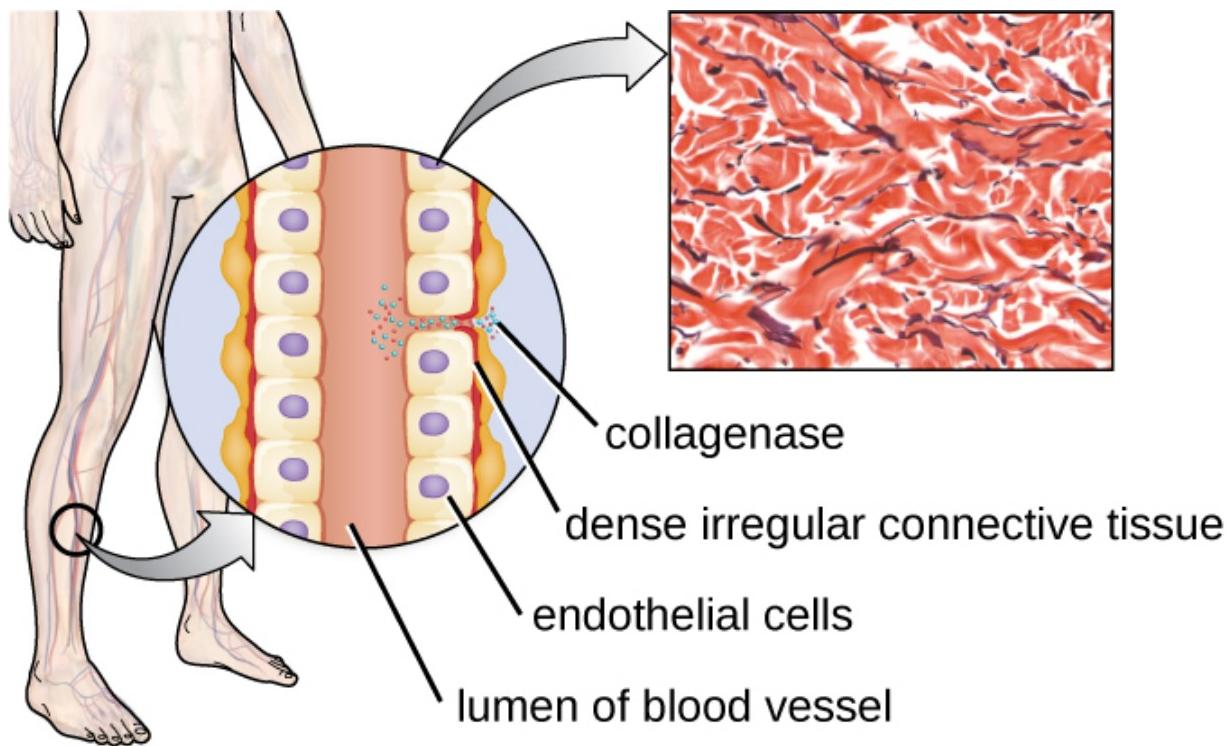
Pathogen-produced nucleases, such as **DNAse** produced by *S. aureus*, degrade extracellular DNA as a means of escape and spreading through tissue. As bacterial and host cells die at the site of infection, they lyse and release their intracellular contents. The DNA chromosome is the largest of the intracellular molecules, and masses of extracellular DNA can trap bacteria and prevent their spread. *S. aureus*

produces a DNase to degrade the mesh of extracellular DNA so it can escape and spread to adjacent tissues. This strategy is also used by *S. aureus* and other pathogens to degrade and escape webs of extracellular DNA produced by immune system phagocytes to trap the bacteria.

Enzymes that degrade the phospholipids of cell membranes are called phospholipases. Their actions are specific in regard to the type of phospholipids they act upon and where they enzymatically cleave the molecules. The pathogen responsible for anthrax, *B. anthracis*, produces phospholipase C. When *B. anthracis* is ingested by phagocytic cells of the immune system, phospholipase C degrades the membrane of the phagosome before it can fuse with the lysosome, allowing the pathogen to escape into the cytoplasm and multiply. Phospholipases can also target the membrane that encloses the phagosome within phagocytic cells. As described earlier in this chapter, this is the mechanism used by intracellular pathogens such as *L. monocytogenes* and *Rickettsia* to escape the phagosome and multiply within the cytoplasm of phagocytic cells. The role of phospholipases in bacterial virulence is not restricted to phagosomal escape. Many pathogens produce phospholipases that act to degrade cell membranes and cause lysis of target cells. These phospholipases are involved in lysis of red blood cells, white blood cells, and tissue cells.

Bacterial pathogens also produce various protein-digesting enzymes, or proteases. Proteases can be classified according to their substrate target (e.g., serine proteases target proteins with the amino acid serine) or if they contain metals in their active site (e.g., zinc metalloproteases contain a zinc ion, which is necessary for enzymatic activity).

One example of a protease that contains a metal ion is the exoenzyme **collagenase**. Collagenase digests collagen, the dominant protein in connective tissue. Collagen can be found in the extracellular matrix, especially near mucosal membranes, blood vessels, nerves, and in the layers of the skin. Similar to hyaluronidase, collagenase allows the pathogen to penetrate and spread through the host tissue by digesting this connective tissue protein. The collagenase produced by the gram-positive bacterium *Clostridium perfringens*, for example, allows the bacterium to make its way through the tissue layers and subsequently enter and multiply in the blood (septicemia). *C. perfringens* then uses toxins and a phospholipase to cause cellular lysis and necrosis. Once the host cells have died, the bacterium produces gas by fermenting the muscle carbohydrates. The widespread necrosis of tissue and accompanying gas are characteristic of the condition known as gas gangrene ([\[link\]](#)).



The illustration depicts a blood vessel with a single layer of endothelial cells surrounding the lumen and dense connective tissue (shown in red) surrounding the endothelial cell layer. Collagenase produced by *C. perfringens* degrades the collagen between the endothelial cells, allowing the bacteria to enter the bloodstream. (credit illustration: modification of work by Bruce Blaus; credit micrograph: Micrograph provided by the Regents of University of Michigan Medical School © 2012)

Note:

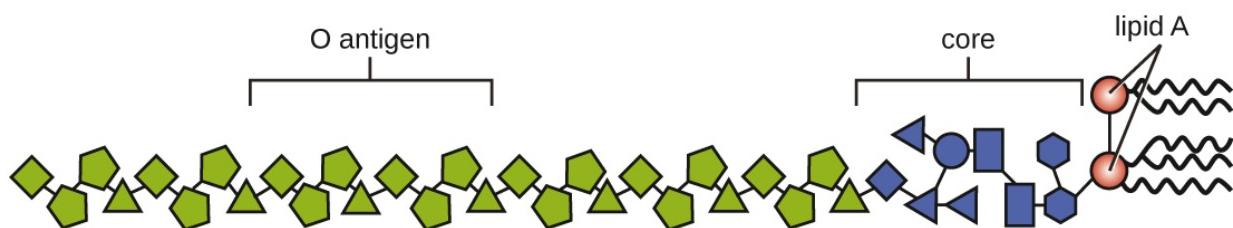


Two types of cell death are apoptosis and necrosis. Visit this [website](#) to learn more about the differences between these mechanisms of cell death and their causes.

Toxins

In addition to exoenzymes, certain pathogens are able to produce **toxins**, biological poisons that assist in their ability to invade and cause damage to tissues. The ability of a pathogen to produce toxins to cause damage to host cells is called **toxigenicity**.

Toxins can be categorized as endotoxins or exotoxins. The lipopolysaccharide (LPS) found on the outer membrane of gram-negative bacteria is called **endotoxin** ([\[link\]](#)). During infection and disease, gram-negative bacterial pathogens release endotoxin either when the cell dies, resulting in the disintegration of the membrane, or when the bacterium undergoes binary fission. The lipid component of endotoxin, lipid A, is responsible for the toxic properties of the LPS molecule. Lipid A is relatively conserved across different genera of gram-negative bacteria; therefore, the toxic properties of lipid A are similar regardless of the gram-negative pathogen. In a manner similar to that of tumor necrosis factor, lipid A triggers the immune system's inflammatory response (see [Inflammation and Fever](#)). If the concentration of endotoxin in the body is low, the inflammatory response may provide the host an effective defense against infection; on the other hand, high concentrations of endotoxin in the blood can cause an excessive inflammatory response, leading to a severe drop in blood pressure, multi-organ failure, and death.



Lipopolysaccharide is composed of lipid A, a core glycolipid, and an O-specific polysaccharide side chain. Lipid A is the toxic component that promotes inflammation and fever.

A classic method of detecting endotoxin is by using the *Limulus* amebocyte lysate (LAL) test. In this procedure, the blood cells (amebocytes) of the horseshoe crab

(*Limulus polyphemus*) is mixed with a patient's serum. The amebocytes will react to the presence of any endotoxin. This reaction can be observed either chromogenically (color) or by looking for coagulation (clotting reaction) to occur within the serum. An alternative method that has been used is an enzyme-linked immunosorbent assay (ELISA) that uses antibodies to detect the presence of endotoxin.

Unlike the toxic lipid A of endotoxin, **exotoxins** are protein molecules that are produced by a wide variety of living pathogenic bacteria. Although some gram-negative pathogens produce exotoxins, the majority are produced by gram-positive pathogens. Exotoxins differ from endotoxin in several other key characteristics, summarized in [link]. In contrast to endotoxin, which stimulates a general systemic inflammatory response when released, exotoxins are much more specific in their action and the cells they interact with. Each exotoxin targets specific receptors on specific cells and damages those cells through unique molecular mechanisms.

Endotoxin remains stable at high temperatures, and requires heating at 121 °C (250 °F) for 45 minutes to inactivate. By contrast, most exotoxins are heat labile because of their protein structure, and many are denatured (inactivated) at temperatures above 41 °C (106 °F). As discussed earlier, endotoxin can stimulate a lethal inflammatory response at very high concentrations and has a measured LD₅₀ of 0.24 mg/kg. By contrast, very small concentrations of exotoxins can be lethal. For example, botulinum toxin, which causes botulism, has an LD₅₀ of 0.000001 mg/kg (240,000 times more lethal than endotoxin).

Comparison of Endotoxin and Exotoxins Produced by Bacteria

Characteristic	Endotoxin	Exotoxin
Source	Gram-negative bacteria	Gram-positive (primarily) and gram-negative bacteria
Composition	Lipid A component of lipopolysaccharide	Protein

Comparison of Endotoxin and Exotoxins Produced by Bacteria

Characteristic	Endotoxin	Exotoxin
Effect on host	General systemic symptoms of inflammation and fever	Specific damage to cells dependent upon receptor-mediated targeting of cells and specific mechanisms of action
Heat stability	Heat stable	Most are heat labile, but some are heat stable
LD ₅₀	High	Low

The exotoxins can be grouped into three categories based on their target: intracellular targeting, membrane disrupting, and superantigens. [\[link\]](#) provides examples of well-characterized toxins within each of these three categories.

Some Common Exotoxins and Associated Bacterial Pathogens

Category	Example	Pathogen	Mechanism and Disease

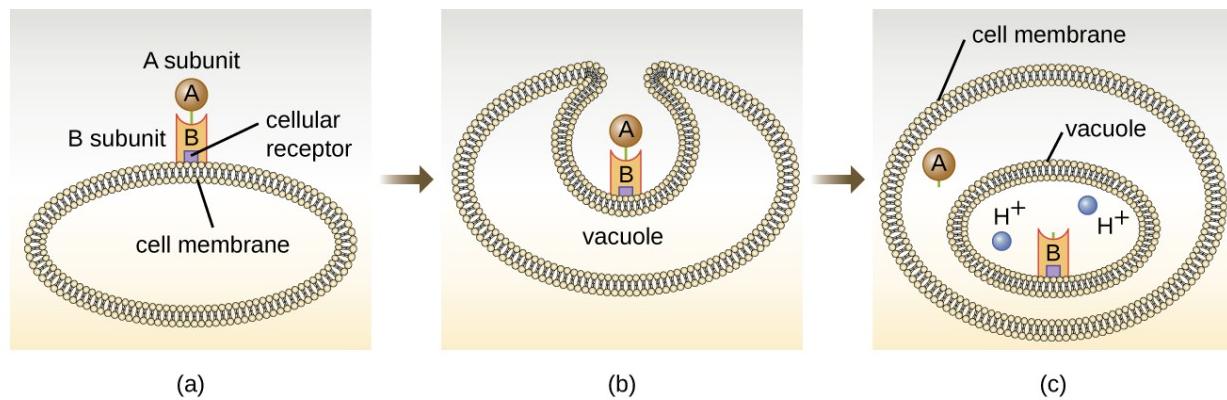
Some Common Exotoxins and Associated Bacterial Pathogens

Category	Example	Pathogen	Mechanism and Disease
Intracellular-targeting toxins	Cholera toxin	<i>Vibrio cholerae</i>	Activation of adenylate cyclase in intestinal cells, causing increased levels of cyclic adenosine monophosphate (cAMP) and secretion of fluids and electrolytes out of cell, causing diarrhea
	Tetanus toxin	<i>Clostridium tetani</i>	Inhibits the release of inhibitory neurotransmitters in the central nervous system, causing spastic paralysis
	Botulinum toxin	<i>Clostridium botulinum</i>	Inhibits release of the neurotransmitter acetylcholine from neurons, resulting in flaccid paralysis

Some Common Exotoxins and Associated Bacterial Pathogens			
Category	Example	Pathogen	Mechanism and Disease
Membrane-disrupting toxins	Diphtheria toxin	<i>Corynebacterium diphtheriae</i>	Inhibition of protein synthesis, causing cellular death
	Streptolysin	<i>Streptococcus pyogenes</i>	Proteins that assemble into pores in cell membranes, disrupting their function and killing the cell
	Pneumolysin	<i>Streptococcus pneumoniae</i>	
	Alpha-toxin	<i>Staphylococcus aureus</i>	
	Alpha-toxin	<i>Clostridium perfringens</i>	Phospholipases that degrade cell membrane phospholipids, disrupting membrane function and killing the cell
	Phospholipase C	<i>Pseudomonas aeruginosa</i>	
Superantigens	Beta-toxin	<i>Staphylococcus aureus</i>	
	Toxic shock syndrome toxin	<i>Staphylococcus aureus</i>	Stimulates excessive activation of immune system cells and release of cytokines (chemical mediators) from immune system cells. Life-threatening fever, inflammation,
	Streptococcal mitogenic exotoxin	<i>Streptococcus pyogenes</i>	

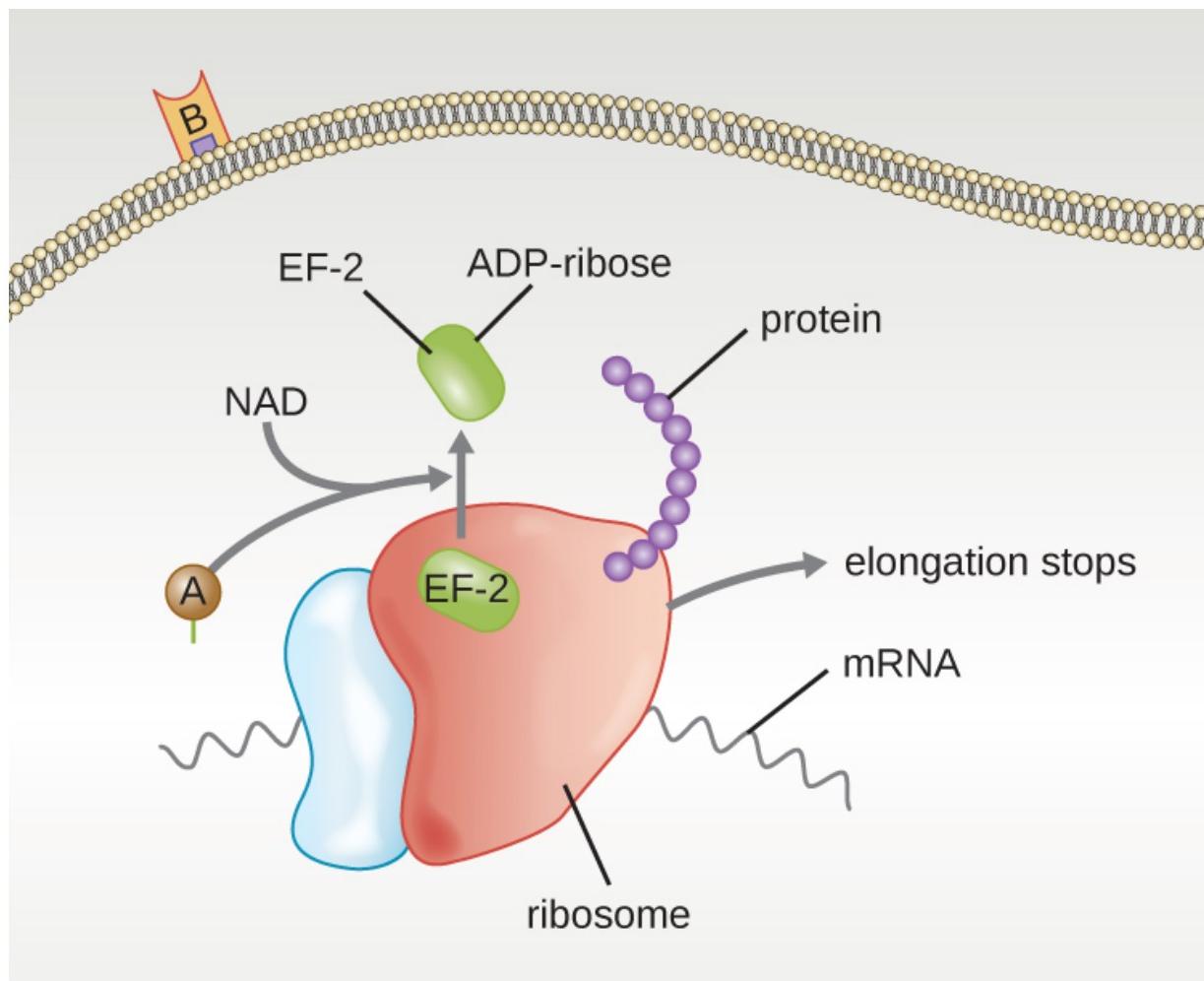
Some Common Exotoxins and Associated Bacterial Pathogens			and shock are the result.
Category	Example	Pathogen	Mechanism and Disease
	Streptococcal pyrogenic toxins	<i>Streptococcus pyogenes</i>	

The **intracellular targeting toxins** comprise two components: A for activity and B for binding. Thus, these types of toxins are known as **A-B exotoxins** ([\[link\]](#)). The B component is responsible for the cellular specificity of the toxin and mediates the initial attachment of the toxin to specific cell surface receptors. Once the A-B toxin binds to the host cell, it is brought into the cell by endocytosis and entrapped in a vacuole. The A and B subunits separate as the vacuole acidifies. The A subunit then enters the cell cytoplasm and interferes with the specific internal cellular function that it targets.



- (a) In A-B toxins, the B component binds to the host cell through its interaction with specific cell surface receptors. (b) The toxin is brought in through endocytosis. (c) Once inside the vacuole, the A component (active component) separates from the B component and the A component gains access to the cytoplasm. (credit: modification of work by “Biology Discussion Forum”/YouTube)

Four unique examples of A-B toxins are the diphtheria, cholera, botulinum, and tetanus toxins. The diphtheria toxin is produced by the gram-positive bacterium *Corynebacterium diphtheriae*, the causative agent of nasopharyngeal and cutaneous diphtheria. After the A subunit of the diphtheria toxin separates and gains access to the cytoplasm, it facilitates the transfer of adenosine diphosphate (ADP)-ribose onto an elongation-factor protein (EF-2) that is needed for protein synthesis. Hence, diphtheria toxin inhibits protein synthesis in the host cell, ultimately killing the cell ([\[link\]](#)).



The mechanism of the diphtheria toxin inhibiting protein synthesis. The A subunit inactivates elongation factor 2 by transferring an ADP-ribose. This stops protein elongation, inhibiting protein synthesis and killing the cell.

Cholera toxin is an **enterotoxin** produced by the gram-negative bacterium *Vibrio cholerae* and is composed of one A subunit and five B subunits. The mechanism of action of the cholera toxin is complex. The B subunits bind to receptors on the intestinal epithelial cell of the small intestine. After gaining entry into the cytoplasm of the epithelial cell, the A subunit activates an intracellular G protein. The activated G protein, in turn, leads to the activation of the enzyme adenyl cyclase, which begins to produce an increase in the concentration of cyclic AMP (a secondary messenger molecule). The increased cAMP disrupts the normal physiology of the intestinal epithelial cells and causes them to secrete excessive amounts of fluid and electrolytes into the lumen of the intestinal tract, resulting in severe “rice-water stool” diarrhea characteristic of cholera.

Botulinum toxin (also known as botox) is a neurotoxin produced by the gram-positive bacterium *Clostridium botulinum*. It is the most acutely toxic substance known to date. The toxin is composed of a light A subunit and heavy protein chain B subunit. The B subunit binds to neurons to allow botulinum toxin to enter the neurons at the neuromuscular junction. The A subunit acts as a protease, cleaving proteins involved in the neuron’s release of acetylcholine, a neurotransmitter molecule. Normally, neurons release acetylcholine to induce muscle fiber contractions. The toxin’s ability to block acetylcholine release results in the inhibition of muscle contractions, leading to muscle relaxation. This has the potential to stop breathing and cause death. Because of its action, low concentrations of botox are used for cosmetic and medical procedures, including the removal of wrinkles and treatment of overactive bladder.

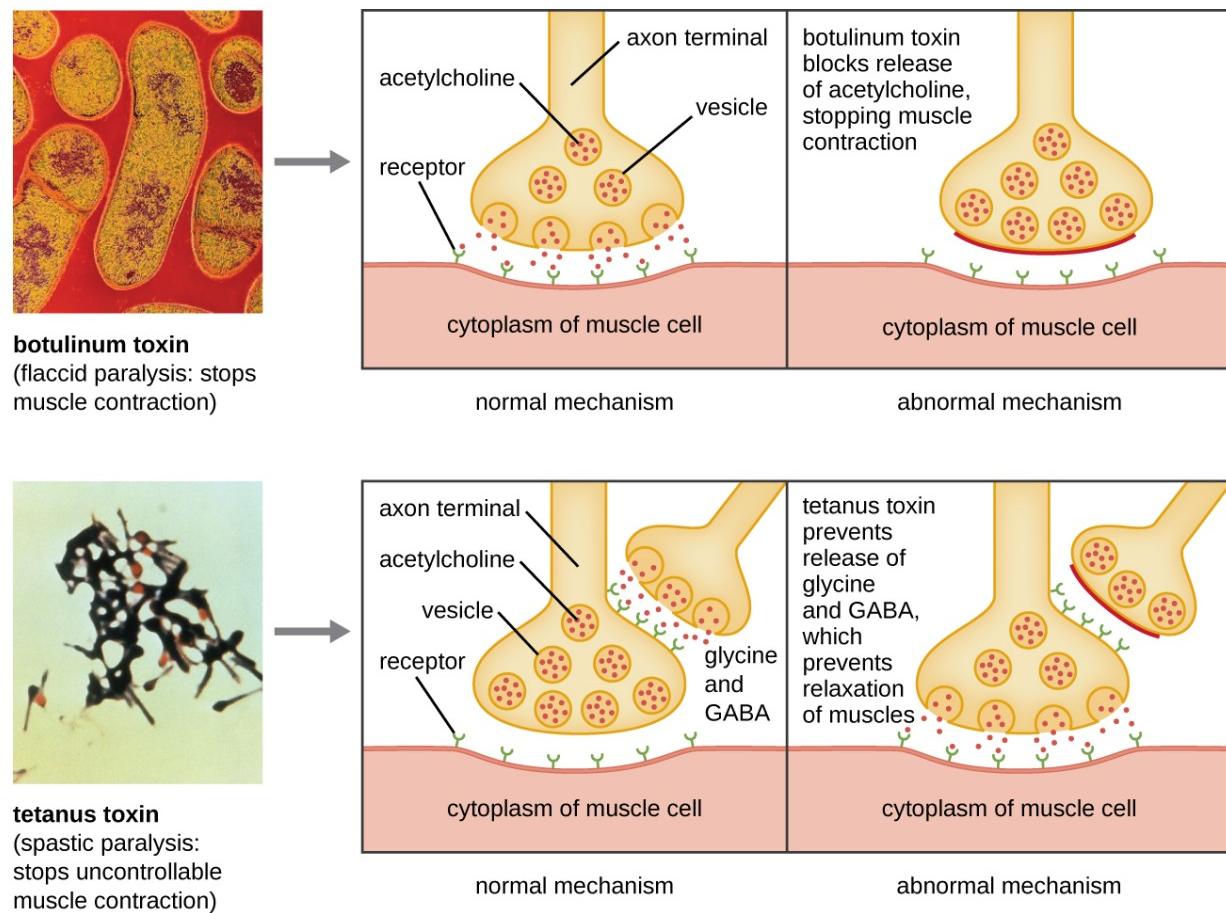
Note:



Click this [link](#) to see an animation of how the cholera toxin functions.

Click this [link](#) to see an animation of how the botulinum toxin functions.

Another neurotoxin is tetanus toxin, which is produced by the gram-positive bacterium *Clostridium tetani*. This toxin also has a light A subunit and heavy protein chain B subunit. Unlike botulinum toxin, tetanus toxin binds to inhibitory interneurons, which are responsible for release of the inhibitory neurotransmitters glycine and gamma-aminobutyric acid (GABA). Normally, these neurotransmitters bind to neurons at the neuromuscular junction, resulting in the inhibition of acetylcholine release. Tetanus toxin inhibits the release of glycine and GABA from the interneuron, resulting in permanent muscle contraction. The first symptom is typically stiffness of the jaw (lockjaw). Violent muscle spasms in other parts of the body follow, typically culminating with respiratory failure and death. [\[link\]](#) shows the actions of both botulinum and tetanus toxins.



Mechanisms of botulinum and tetanus toxins. (credit micrographs: modification of work by Centers for Disease Control and Prevention)

Membrane-disrupting toxins affect cell membrane function either by forming pores or by disrupting the phospholipid bilayer in host cell membranes. Two types of membrane-disrupting exotoxins are **hemolysins** and leukocidins, which form pores in cell membranes, causing leakage of the cytoplasmic contents and cell lysis. These toxins were originally thought to target red blood cells (erythrocytes) and white blood cells (leukocytes), respectively, but we now know they can affect other cells as well. The gram-positive bacterium *Streptococcus pyogenes* produces streptolysins, water-soluble hemolysins that bind to the cholesterol moieties in the host cell membrane to form a pore. The two types of streptolysins, O and S, are categorized by their ability to cause hemolysis in erythrocytes in the absence or presence of oxygen. Streptolysin O is not active in the presence of oxygen, whereas streptolysin S is active in the presence of oxygen. Other important pore-forming membrane-disrupting toxins include alpha toxin of *Staphylococcus aureus* and pneumolysin of *Streptococcus pneumoniae*.

Bacterial phospholipases are **membrane-disrupting toxins** that degrade the phospholipid bilayer of cell membranes rather than forming pores. We have already discussed the phospholipases associated with *B. anthracis*, *L. pneumophila*, and *Rickettsia* species that enable these bacteria to effect the lysis of phagosomes. These same phospholipases are also hemolysins. Other phospholipases that function as hemolysins include the alpha toxin of *Clostridium perfringens*, phospholipase C of *P. aeruginosa*, and beta toxin of *Staphylococcus aureus*.

Some strains of *S. aureus* also produce a leukocidin called Panton-Valentine leukocidin (PVL). PVL consists of two subunits, S and F. The S component acts like the B subunit of an A-B exotoxin in that it binds to glycolipids on the outer plasma membrane of animal cells. The F-component acts like the A subunit of an A-B exotoxin and carries the enzymatic activity. The toxin inserts and assembles into a pore in the membrane. Genes that encode PVL are more frequently present in *S. aureus* strains that cause skin infections and pneumonia.[\[footnote\]](#) PVL promotes skin infections by causing edema, erythema (reddening of the skin due to blood vessel dilation), and skin necrosis. PVL has also been shown to cause necrotizing pneumonia. PVL promotes pro-inflammatory and cytotoxic effects on alveolar leukocytes. This results in the release of enzymes from the leukocytes, which, in turn, cause damage to lung tissue.

V. Meka. “Panton-Valentine Leukocidin.”

<http://www.antimicrobe.org/h04c.files/history/PVL-S-aureus.asp>

The third class of exotoxins is the **superantigens**. These are exotoxins that trigger an excessive, nonspecific stimulation of immune cells to secrete cytokines (chemical messengers). The excessive production of cytokines, often called a cytokine storm, elicits a strong immune and inflammatory response that can cause life-threatening

high fevers, low blood pressure, multi-organ failure, shock, and death. The prototype superantigen is the toxic shock syndrome toxin of *S. aureus*. Most toxic shock syndrome cases are associated with vaginal colonization by toxin-producing *S. aureus* in menstruating women; however, colonization of other body sites can also occur. Some strains of *Streptococcus pyogenes* also produce superantigens; they are referred to as the streptococcal mitogenic exotoxins and the streptococcal pyrogenic toxins.

Note:

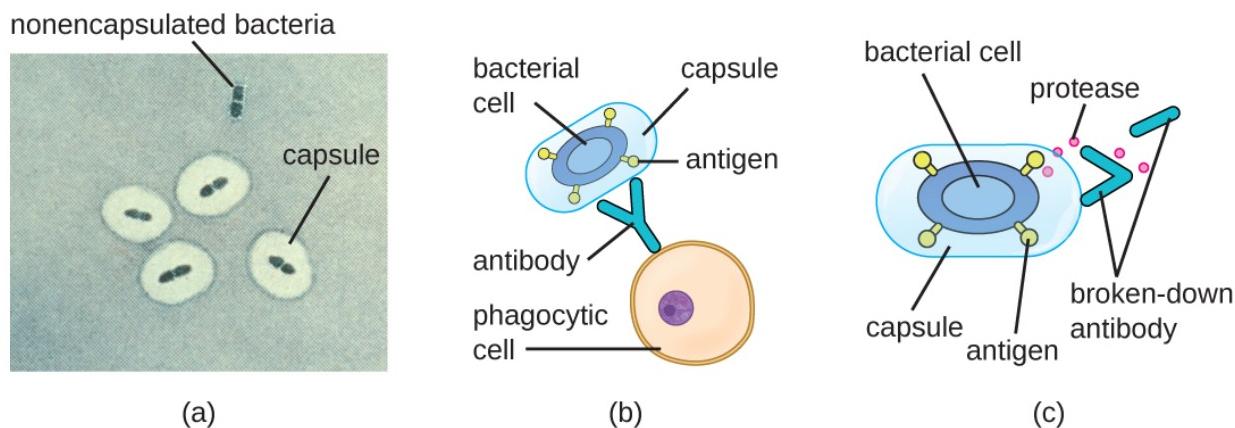
- Describe how exoenzymes contribute to bacterial invasion.
- Explain the difference between exotoxins and endotoxin.
- Name the three classes of exotoxins.

Virulence Factors for Survival in the Host and Immune Evasion

Evading the immune system is also important to invasiveness. Bacteria use a variety of virulence factors to evade phagocytosis by cells of the immune system. For example, many bacteria produce capsules, which are used in adhesion but also aid in immune evasion by preventing ingestion by phagocytes. The composition of the capsule prevents immune cells from being able to adhere and then phagocytose the cell. In addition, the capsule makes the bacterial cell much larger, making it harder for immune cells to engulf the pathogen ([\[link\]](#)). A notable capsule-producing bacterium is the gram-positive pathogen *Streptococcus pneumoniae*, which causes pneumococcal pneumonia, meningitis, septicemia, and other respiratory tract infections. Encapsulated strains of *S. pneumoniae* are more virulent than nonencapsulated strains and are more likely to invade the bloodstream and cause septicemia and meningitis.

Some pathogens can also produce proteases to protect themselves against phagocytosis. As described in [Adaptive Specific Host Defenses](#), the human immune system produces antibodies that bind to surface molecules found on specific bacteria (e.g., capsules, fimbriae, flagella, LPS). This binding initiates phagocytosis and other mechanisms of antibacterial killing and clearance. Proteases combat antibody-mediated killing and clearance by attacking and digesting the antibody molecules ([\[link\]](#)).

In addition to capsules and proteases, some bacterial pathogens produce other virulence factors that allow them to evade the immune system. The fimbriae of certain species of *Streptococcus* contain M protein, which alters the surface of *Streptococcus* and inhibits phagocytosis by blocking the binding of the complement molecules that assist phagocytes in ingesting bacterial pathogens. The acid-fast bacterium *Mycobacterium tuberculosis* (the causative agent of tuberculosis) produces a waxy substance known as mycolic acid in its cell envelope. When it is engulfed by phagocytes in the lung, the protective mycolic acid coat enables the bacterium to resist some of the killing mechanisms within the phagolysosome.



(a) A micrograph of capsules around bacterial cells. (b) Antibodies normally function by binding to antigens, molecules on the surface of pathogenic bacteria. Phagocytes then bind to the antibody, initiating phagocytosis. (c) Some bacteria also produce proteases, virulence factors that break down host antibodies to evade phagocytosis. (credit a: modification of work by Centers for Disease Control and Prevention)

Some bacteria produce virulence factors that promote infection by exploiting molecules naturally produced by the host. For example, most strains of *Staphylococcus aureus* produce the exoenzyme **coagulase**, which exploits the natural mechanism of blood clotting to evade the immune system. Normally, blood clotting is triggered in response to blood vessel damage; platelets begin to plug the clot, and a cascade of reactions occurs in which fibrinogen, a soluble protein made by the liver, is cleaved into fibrin. Fibrin is an insoluble, thread-like protein that binds to blood platelets, cross-links, and contracts to form a mesh of clumped platelets and red blood cells. The resulting clot prevents further loss of blood from

the damaged blood vessels. However, if bacteria release coagulase into the bloodstream, the fibrinogen-to-fibrin cascade is triggered in the absence of blood vessel damage. The resulting clot coats the bacteria in fibrin, protecting the bacteria from exposure to phagocytic immune cells circulating in the bloodstream.

Whereas coagulase causes blood to clot, kinases have the opposite effect by triggering the conversion of plasminogen to plasmin, which is involved in the digestion of fibrin clots. By digesting a clot, kinases allow pathogens trapped in the clot to escape and spread, similar to the way that collagenase, hyaluronidase, and DNase facilitate the spread of infection. Examples of kinases include staphylokinases and streptokinases, produced by *Staphylococcus aureus* and *Streptococcus pyogenes*, respectively. It is intriguing that *S. aureus* can produce both coagulase to promote clotting and staphylokinase to stimulate the digestion of clots. The action of the coagulase provides an important protective barrier from the immune system, but when nutrient supplies are diminished or other conditions signal a need for the pathogen to escape and spread, the production of staphylokinase can initiate this process.

A final mechanism that pathogens can use to protect themselves against the immune system is called **antigenic variation**, which is the alteration of surface proteins so that a pathogen is no longer recognized by the host's immune system. For example, the bacterium *Borrelia burgdorferi*, the causative agent of Lyme disease, contains a surface lipoprotein known as VlsE. Because of genetic recombination during DNA replication and repair, this bacterial protein undergoes antigenic variation. Each time fever occurs, the VlsE protein in *B. burgdorferi* can differ so much that antibodies against previous VlsE sequences are not effective. It is believed that this variation in the VlsE contributes to the ability *B. burgdorferi* to cause chronic disease. Another important human bacterial pathogen that uses antigenic variation to avoid the immune system is *Neisseria gonorrhoeae*, which causes the sexually transmitted disease gonorrhea. This bacterium is well known for its ability to undergo antigenic variation of its type IV pili to avoid immune defenses.

Note:

- Name at least two ways that a capsule provides protection from the immune system.
- Besides capsules, name two other virulence factors used by bacteria to evade the immune system.

Viral Virulence

Although viral pathogens are not similar to bacterial pathogens in terms of structure, some of the properties that contribute to their virulence are similar. Viruses use adhesins to facilitate adhesion to host cells, and certain enveloped viruses rely on antigenic variation to avoid the host immune defenses. These virulence factors are discussed in more detail in the following sections.

Viral Adhesins

One of the first steps in any viral infection is adhesion of the virus to specific receptors on the surface of cells. This process is mediated by adhesins that are part of the viral capsid or membrane envelope. The interaction of viral adhesins with specific cell receptors defines the tropism (preferential targeting) of viruses for specific cells, tissues, and organs in the body. The spike protein hemagglutinin found on Influenzavirus is an example of a viral adhesin; it allows the virus to bind to the sialic acid on the membrane of host respiratory and intestinal cells. Another viral adhesin is the glycoprotein gp20, found on HIV. For HIV to infect cells of the immune system, it must interact with two receptors on the surface of cells. The first interaction involves binding between gp120 and the CD4 cellular marker that is found on some essential immune system cells. However, before viral entry into the cell can occur, a second interaction between gp120 and one of two chemokine receptors (CCR5 and CXCR4) must occur. [\[link\]](#) lists the adhesins for some common viral pathogens and the specific sites to which these adhesins allow viruses to attach.

Some Viral Adhesins and Their Host Attachment Sites

Pathogen	Disease	Adhesin	Attachment Site
Influenzavirus	Influenza	Hemagglutinin	Sialic acid of respiratory and intestinal cells

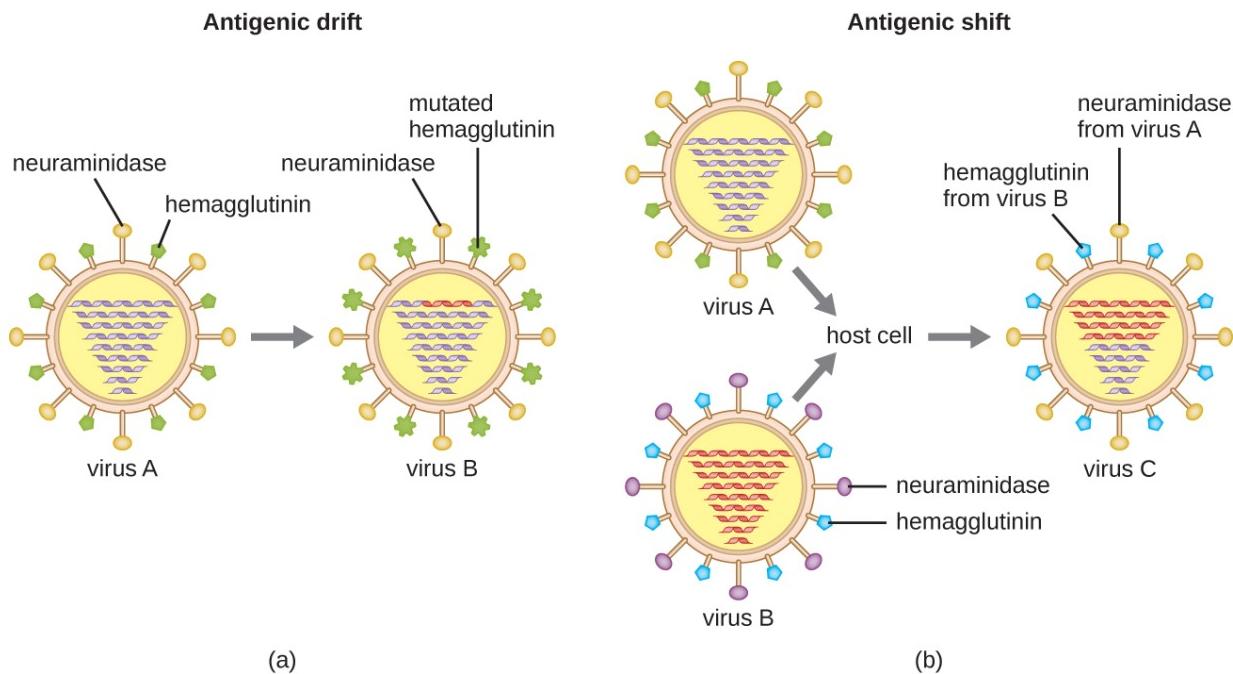
Some Viral Adhesins and Their Host Attachment Sites

Pathogen	Disease	Adhesin	Attachment Site
Herpes simplex virus I or II	Oral herpes, genital herpes	Glycoproteins gB, gC, gD	Heparan sulfate on mucosal surfaces of the mouth and genitals
Human immunodeficiency virus	HIV/AIDS	Glycoprotein gp120	CD4 and CCR5 or CXCR4 of immune system cells

Antigenic Variation in Viruses

Antigenic variation also occurs in certain types of enveloped viruses, including influenza viruses, which exhibit two forms of antigenic variation: **antigenic drift** and **antigenic shift** ([\[link\]](#)). Antigenic drift is the result of point mutations causing slight changes in the spike proteins hemagglutinin (H) and neuraminidase (N). On the other hand, antigenic shift is a major change in spike proteins due to gene reassortment. This reassortment for antigenic shift occurs typically when two different influenza viruses infect the same host.

The rate of antigenic variation in influenza viruses is very high, making it difficult for the immune system to recognize the many different strains of Influenzavirus. Although the body may develop immunity to one strain through natural exposure or vaccination, antigenic variation results in the continual emergence of new strains that the immune system will not recognize. This is the main reason that vaccines against Influenzavirus must be given annually. Each year's influenza vaccine provides protection against the most prevalent strains for that year, but new or different strains may be more prevalent the following year.



Antigenic drift and antigenic shift in influenza viruses. (a) In antigenic drift, mutations in the genes for the surface proteins neuraminidase and/or hemagglutinin result in small antigenic changes over time. (b) In antigenic shift, simultaneous infection of a cell with two different influenza viruses results in mixing of the genes. The resultant virus possesses a mixture of the proteins of the original viruses. Influenza pandemics can often be traced to antigenic shifts.

Note:



For another explanation of how [antigenic shift and drift](#) occur, watch this video.

Note:

- Describe the role of adhesins in viral tropism.
- Explain the difference between antigenic drift and antigenic shift.

Key Concepts and Summary

- **Virulence factors** contribute to a pathogen's ability to cause disease.
- **Exoenzymes** and **toxins** allow pathogens to invade host tissue and cause tissue damage. Exoenzymes are classified according to the macromolecule they target and exotoxins are classified based on their mechanism of action.
- Bacterial toxins include **endotoxin** and **exotoxins**. Endotoxin is the lipid A component of the LPS of the gram-negative cell envelope. Exotoxins are proteins secreted mainly by gram-positive bacteria, but also are secreted by gram-negative bacteria.
- Bacterial pathogens may evade the host immune response by producing **capsules** to avoid phagocytosis, surviving the intracellular environment of phagocytes, degrading antibodies, or through **antigenic variation**.
- Viral pathogens use adhesins for initiating infections and antigenic variation to avoid immune defenses.
- Influenza viruses use both **antigenic drift** and **antigenic shift** to avoid being recognized by the immune system.

Critical Thinking

Exercise:**Problem:**

Imagine that a mutation in the gene encoding the cholera toxin was made. This mutation affects the A-subunit, preventing it from interacting with any host protein. (a) Would the toxin be able to enter into the intestinal epithelial cell? (b) Would the toxin be able to cause diarrhea?

Virulence Factors of Eukaryotic Pathogens

LEARNING OBJECTIVES

- Describe virulence factors unique to fungi and parasites
- Compare virulence factors of fungi and bacteria
- Explain the difference between protozoan parasites and helminths
- Describe how helminths evade the host immune system

Although fungi and parasites are important pathogens causing infectious diseases, their pathogenic mechanisms and virulence factors are not as well characterized as those of bacteria. Despite the relative lack of detailed mechanisms, the stages of pathogenesis and general mechanisms of virulence involved in disease production by these pathogens are similar to those of bacteria.

Fungal Virulence

Pathogenic fungi can produce virulence factors that are similar to the bacterial virulence factors that have been discussed earlier in this chapter. In this section, we will look at the virulence factors associated with species of *Candida*, *Cryptococcus*, *Claviceps*, and *Aspergillus*.

Candida albicans is an opportunistic fungal pathogen and causative agent of oral thrush, vaginal yeast infections, and cutaneous candidiasis. *Candida* produces adhesins (surface glycoproteins) that bind to the phospholipids of epithelial and endothelial cells. To assist in spread and tissue invasion, *Candida* produces proteases and phospholipases (i.e., exoenzymes). One of

these proteases degrades keratin, a structural protein found on epithelial cells, enhancing the ability of the fungus to invade host tissue. In animal studies, it has been shown that the addition of a protease inhibitor led to attenuation of *Candida* infection.[\[footnote\]](#) Similarly, the phospholipases can affect the integrity of host cell membranes to facilitate invasion.

K. Fallon et al. “Role of Aspartic Proteases in Disseminated *Candida albicans* Infection in Mice.” *Infection and Immunity* 65 no. 2 (1997):551–556.

The main virulence factor for *Cryptococcus*, a fungus that causes pneumonia and meningitis, is capsule production. The polysaccharide glucuronoxylomannan is the principal constituent of the *Cryptococcus* capsule. Similar to encapsulated bacterial cells, encapsulated *Cryptococcus* cells are more resistant to phagocytosis than nonencapsulated *Cryptococcus*, which are effectively phagocytosed and, therefore, less virulent.

Like some bacteria, many fungi produce exotoxins. Fungal toxins are called **mycotoxins**. *Claviceps purpurea*, a fungus that grows on rye and related grains, produces a mycotoxin called ergot toxin, an alkaloid responsible for the disease known as ergotism. There are two forms of ergotism: gangrenous and convulsive. In gangrenous ergotism, the ergot toxin causes vasoconstriction, resulting in improper blood flow to the extremities, eventually leading to gangrene. A famous outbreak of gangrenous ergotism occurred in Eastern Europe during the 5th century AD due to the consumption of rye contaminated with *C. purpurea*. In convulsive ergotism, the toxin targets the central nervous system, causing mania and hallucinations.

The mycotoxin aflatoxin is a virulence factor produced by the fungus *Aspergillus*, an opportunistic pathogen that can enter the body via contaminated food or by inhalation. Inhalation of the fungus can lead to the chronic pulmonary disease aspergillosis, characterized by fever, bloody sputum, and/or asthma. Aflatoxin acts in the host as both a mutagen (a substance that causes mutations in DNA) and a **carcinogen** (a substance involved in causing cancer), and has been associated with the development of liver cancer. Aflatoxin has also been shown to cross the blood-placental

barrier.[\[footnote\]](#) A second mycotoxin produced by *Aspergillus* is gliotoxin. This toxin promotes virulence by inducing host cells to self-destruct and by evading the host's immune response by inhibiting the function of phagocytic cells as well as the pro-inflammatory response. Like *Candida*, *Aspergillus* also produces several proteases. One is elastase, which breaks down the protein elastin found in the connective tissue of the lung, leading to the development of lung disease. Another is catalase, an enzyme that protects the fungus from hydrogen peroxide produced by the immune system to destroy pathogens.

C.P. Wild et al. "In-utero exposure to aflatoxin in west Africa." *Lancet* 337 no. 8757 (1991):1602.

Note:

- List virulence factors common to bacteria and fungi.
- What functions do mycotoxins perform to help fungi survive in the host?

Protozoan Virulence

Protozoan pathogens are unicellular eukaryotic parasites that have virulence factors and pathogenic mechanisms analogous to prokaryotic and viral pathogens, including adhesins, toxins, antigenic variation, and the ability to survive inside phagocytic vesicles.

Protozoans often have unique features for attaching to host cells. The protozoan *Giardia lamblia*, which causes the intestinal disease giardiasis, uses a large adhesive disc composed of microtubules to attach to the intestinal mucosa. During adhesion, the flagella of *G. lamblia* move in a manner that draws fluid out from under the disc, resulting in an area of lower pressure that facilitates adhesion to epithelial cells. *Giardia* does not invade the intestinal cells but rather causes inflammation (possibly through

the release of cytopathic substances that cause damage to the cells) and shortens the intestinal villi, inhibiting absorption of nutrients.

Some protozoans are capable of antigenic variation. The obligate intracellular pathogen *Plasmodium falciparum* (one of the causative agents of malaria) resides inside red blood cells, where it produces an adhesin membrane protein known as PfEMP1. This protein is expressed on the surface of the infected erythrocytes, causing blood cells to stick to each other and to the walls of blood vessels. This process impedes blood flow, sometimes leading to organ failure, anemia, jaundice (yellowing of skin and sclera of the eyes due to buildup of bilirubin from lysed red blood cells), and, subsequently, death. Although PfEMP1 can be recognized by the host's immune system, antigenic variations in the structure of the protein over time prevent it from being easily recognized and eliminated. This allows malaria to persist as a chronic infection in many individuals.

The virulence factors of *Trypanosoma brucei*, the causative agent of African sleeping sickness, include the abilities to form capsules and undergo antigenic variation. *T. brucei* evades phagocytosis by producing a dense glycoprotein coat that resembles a bacterial capsule. Over time, host antibodies are produced that recognize this coat, but *T. brucei* is able to alter the structure of the glycoprotein to evade recognition.

Note:

- Explain how antigenic variation by protozoan pathogens helps them survive in the host.

Helminth Virulence

Helminths, or parasitic worms, are multicellular eukaryotic parasites that depend heavily on virulence factors that allow them to gain entry to host tissues. For example, the aquatic larval form of *Schistosoma mansoni*,

which causes schistosomiasis, penetrates intact skin with the aid of proteases that degrade skin proteins, including elastin.

To survive within the host long enough to perpetuate their often-complex life cycles, helminths need to evade the immune system. Some helminths are so large that the immune system is ineffective against them. Others, such as adult roundworms (which cause trichinosis, ascariasis, and other diseases), are protected by a tough outer cuticle.

Over the course of their life cycles, the surface characteristics of the parasites vary, which may help prevent an effective immune response. Some helminths express polysaccharides called glycans on their external surface; because these glycans resemble molecules produced by host cells, the immune system fails to recognize and attack the helminth as a foreign body. This “glycan gimmickry,” as it has been called, serves as a protective cloak that allows the helminth to escape detection by the immune system.

[\[footnote\]](#)

I. van Die, R.D. Cummings. “Glycan Gimmickry by Parasitic Helminths: A Strategy for Modulating the Host Immune Response?” *Glycobiology* 20 no. 1 (2010):2–12.

In addition to evading host defenses, helminths can actively suppress the immune system. *S. mansoni*, for example, degrades host antibodies with proteases. Helminths produce many other substances that suppress elements of both innate nonspecific and adaptive specific host defenses. They also release large amounts of material into the host that may locally overwhelm the immune system or cause it to respond inappropriately.

Note:

- Describe how helminths avoid being destroyed by the host immune system.

Key Concepts and Summary

- Fungal and parasitic pathogens use pathogenic mechanisms and virulence factors that are similar to those of bacterial pathogens
- Fungi initiate infections through the interaction of adhesins with receptors on host cells. Some fungi produce toxins and exoenzymes involved in disease production and capsules that provide protection of phagocytosis.
- Protozoa adhere to target cells through complex mechanisms and can cause cellular damage through release of cytopathic substances. Some protozoa avoid the immune system through antigenic variation and production of capsules.
- Helminthic worms are able to avoid the immune system by coating their exteriors with glycan molecules that make them look like host cells or by suppressing the immune system.

Multiple Choice

Exercise:

Problem:

Which of the following is a major virulence factor for the fungal pathogen *Cryptococcus*?

- A. hemolysin
- B. capsule
- C. collagenase
- D. fimbriae

Solution:

B

Exercise:

Problem:

Which of the following pathogens undergoes antigenic variation to avoid immune defenses?

- A. *Candida*
 - B. *Cryptococcus*
 - C. *Plasmodium*
 - D. *Giardia*
-

Solution:

C

Fill in the Blank**Exercise:****Problem:**

Candida can invade tissue by producing the exoenzymes _____ and _____.

Solution:

protease and phospholipase

Exercise:**Problem:**

The larval form of *Schistosoma mansoni* uses a _____ to help it gain entry through intact skin.

Solution:

protease

Short Answer

Exercise:

Problem:

Describe the virulence factors associated with the fungal pathogen *Aspergillus*.

Exercise:

Problem: Explain how helminths evade the immune system.

Disease and Epidemiology - Introduction

class="introduction"

Signs like
this may
seem self-
explanatory
today, but a
few short
centuries ago,
people lacked
a basic
understandin
g of how
diseases
spread.

Microbiology
has greatly
contributed to
the field of
epidemiology
, which
focuses on
containing
the spread of
disease.

(credit:
modification
of work by
Tony
Webster)



In the United States and other developed nations, public health is a key function of government. A healthy citizenry is more productive, content, and prosperous; high rates of death and disease, on the other hand, can severely hamper economic productivity and foster social and political instability. The burden of disease makes it difficult for citizens to work consistently, maintain employment, and accumulate wealth to better their lives and support a growing economy.

In this chapter, we will explore the intersections between microbiology and epidemiology, the science that underlies public health. Epidemiology studies how disease originates and spreads throughout a population, with the goal of preventing outbreaks and containing them when they do occur. Over the past two centuries, discoveries in epidemiology have led to public health policies that have transformed life in developed nations, leading to the eradication (or near eradication) of many diseases that were once causes of great human suffering and premature death. However, the work of epidemiologists is far from finished. Numerous diseases continue to plague humanity, and new diseases are always emerging. Moreover, in the developing world, lack of infrastructure continues to pose many challenges to efforts to contain disease.

The Language of Epidemiologists

LEARNING OBJECTIVES

- Explain the difference between prevalence and incidence of disease
- Distinguish the characteristics of sporadic, endemic, epidemic, and pandemic diseases
- Explain the use of Koch's postulates and their modifications to determine the etiology of disease
- Explain the relationship between epidemiology and public health

The field of **epidemiology** concerns the geographical distribution and timing of infectious disease occurrences and how they are transmitted and maintained in nature, with the goal of recognizing and controlling outbreaks. The science of epidemiology includes **etiology** (the study of the causes of disease) and investigation of disease transmission (mechanisms by which a disease is spread).

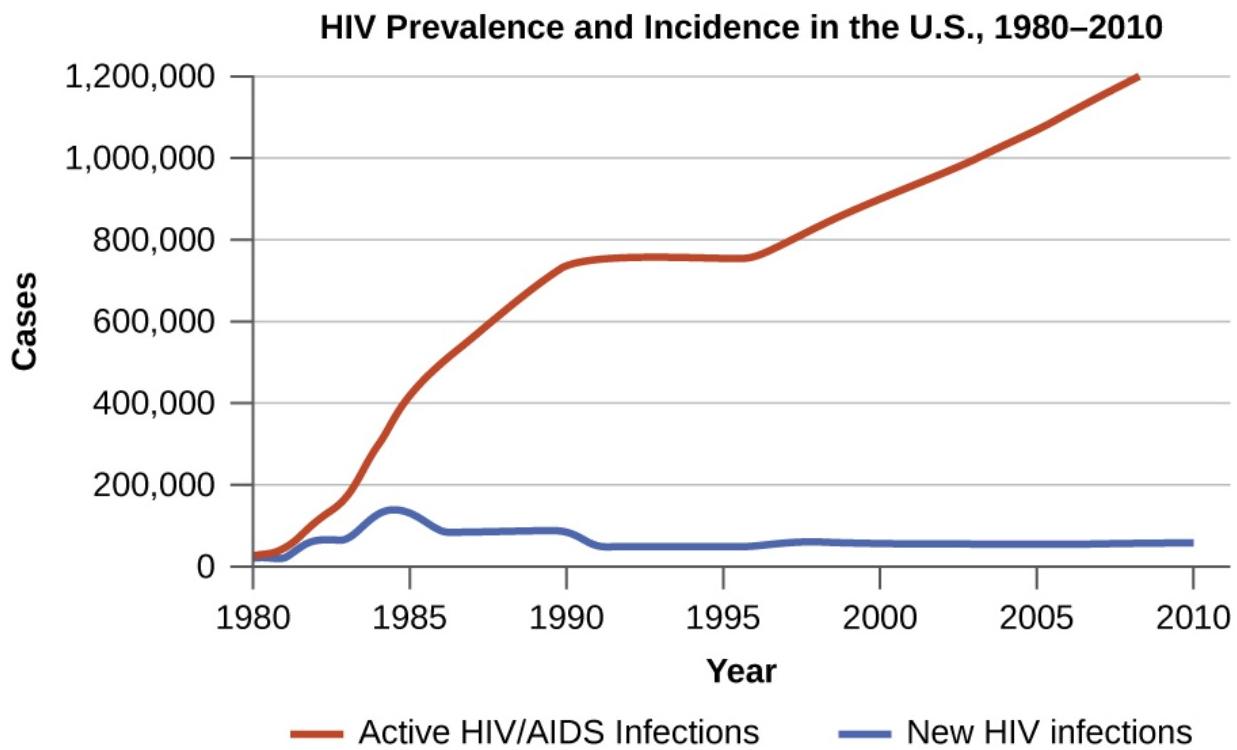
Analyzing Disease in a Population

Epidemiological analyses are always carried out with reference to a population, which is the group of individuals that are at risk for the disease or condition. The population can be defined geographically, but if only a portion of the individuals in that area are susceptible, additional criteria may be required. Susceptible individuals may be defined by particular behaviors, such as intravenous drug use, owning particular pets, or membership in an institution, such as a college. Being able to define the population is important because most measures of interest in epidemiology are made with reference to the size of the population.

The state of being diseased is called **morbidity**. Morbidity in a population can be expressed in a few different ways. Morbidity or total morbidity is expressed in numbers of individuals without reference to the size of the population. The **morbidity rate** can be expressed as the number of diseased individuals out of a standard number of individuals in the population, such as 100,000, or as a percent of the population.

There are two aspects of morbidity that are relevant to an epidemiologist: a disease's **prevalence** and its **incidence**. Prevalence is the number, or proportion, of individuals with a particular illness in a given population at a point in time. For example, the Centers for Disease Control and Prevention (CDC) estimated that in 2012, there were about 1.2 million people 13 years and older with an active human immunodeficiency virus (HIV) infection. Expressed as a proportion, or rate, this is a prevalence of 467 infected persons per 100,000 in the population.[\[footnote\]](#) On the other hand, incidence is the number or proportion of *new* cases in a period of time. For the same year and population, the CDC estimates that there were 43,165 newly diagnosed cases of HIV infection, which is an incidence of 13.7 new cases per 100,000 in the population.[\[footnote\]](#) The relationship between incidence and prevalence can be seen in [\[link\]](#). For a chronic disease like HIV infection, prevalence will generally be higher than incidence because it represents the cumulative number of new cases over many years minus the number of cases that are no longer active (e.g., because the patient died or was cured). H. Irene Hall, Qian An, Tian Tang, Ruiguang Song, Mi Chen, Timothy Green, and Jian Kang. "Prevalence of Diagnosed and Undiagnosed HIV Infection—United States, 2008–2012." *Morbidity and Mortality Weekly Report* 64, no. 24 (2015): 657–662. Centers for Disease Control and Prevention. "Diagnoses of HIV Infection in the United States and Dependent Areas, 2014." *HIV Surveillance Report* 26 (2015).

In addition to morbidity rates, the incidence and prevalence of **mortality** (death) may also be reported. A mortality rate can be expressed as the percentage of the population that has died from a disease or as the number of deaths per 100,000 persons (or other suitable standard number).



This graph compares the incidence of HIV (the number of new cases reported each year) with the prevalence (the total number of cases each year). Prevalence and incidence can also be expressed as a rate or proportion for a given population.

Note:

- Explain the difference between incidence and prevalence.
- Describe how morbidity and mortality rates are expressed.

Patterns of Incidence

Diseases that are seen only occasionally, and usually without geographic concentration, are called **sporadic diseases**. Examples of sporadic diseases include tetanus, rabies, and plague. In the United States, *Clostridium tetani*, the bacterium that causes tetanus, is ubiquitous in the soil environment, but incidences of infection occur only rarely and in scattered locations because most individuals are vaccinated, clean wounds appropriately, or are only rarely in a situation that would cause infection. [footnote]

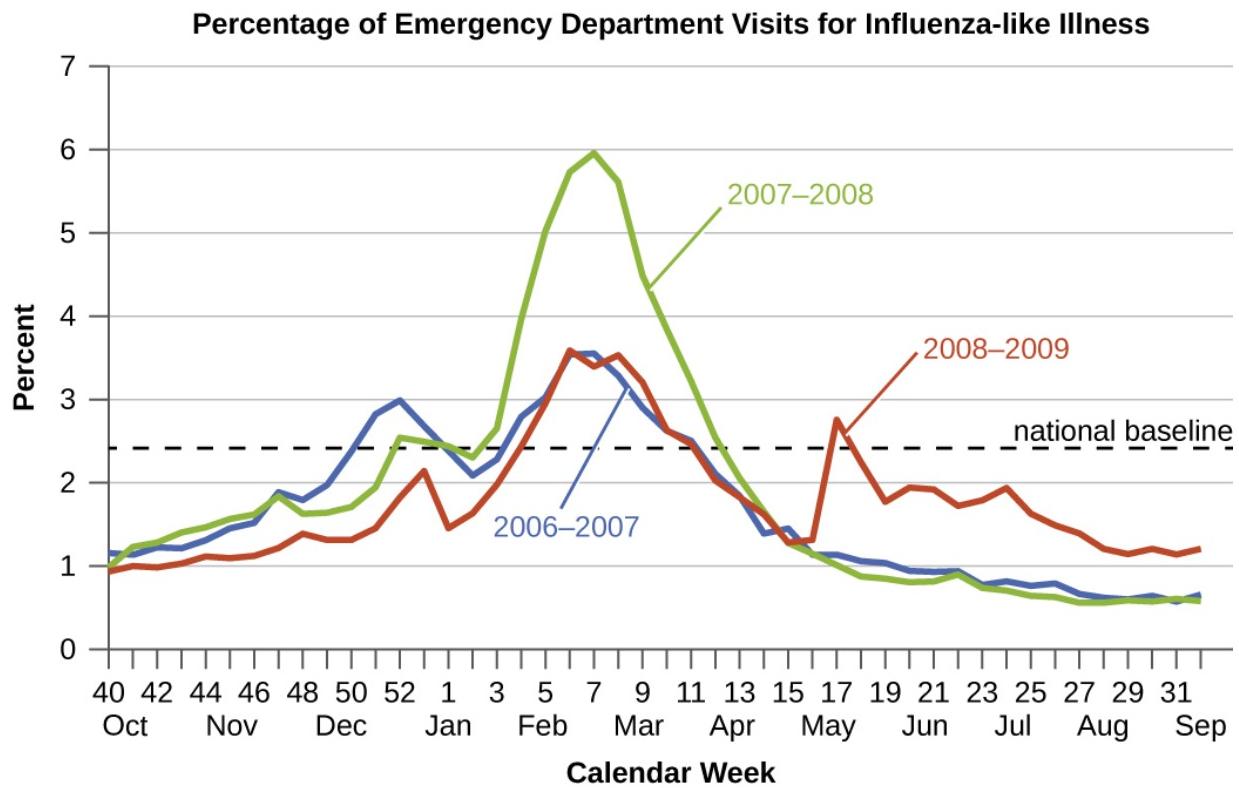
Likewise in the United States there are a few scattered cases of plague each year, usually contracted from rodents in rural areas in the western states.[\[footnote\]](#)
Centers for Disease Control and Prevention. “Tetanus Surveillance—United States, 2001–2008.” *Morbidity and Mortality Weekly Report* 60, no. 12 (2011): 365.
Centers for Disease Control and Prevention. “Plague in the United States.” 2015. <http://www.cdc.gov/plague/maps>. Accessed June 1, 2016.

Diseases that are constantly present (often at a low level) in a population within a particular geographic region are called **endemic diseases**. For example, malaria is endemic to some regions of Brazil, but is not endemic to the United States.

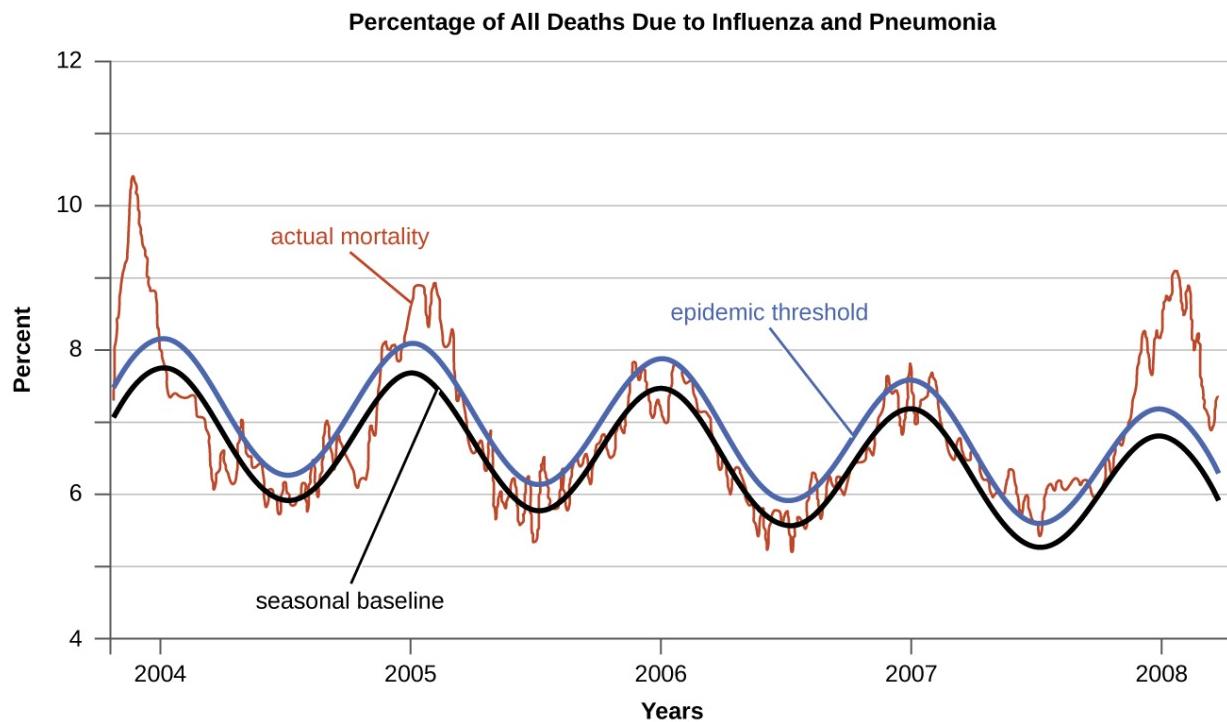
Diseases for which a larger than expected number of cases occurs in a short time within a geographic region are called **epidemic diseases**. Influenza is a good example of a commonly epidemic disease. Incidence patterns of influenza tend to rise each winter in the northern hemisphere. These seasonal increases are expected, so it would not be accurate to say that influenza is epidemic every winter; however, some winters have an unusually large number of seasonal influenza cases in particular regions, and such situations would qualify as epidemics ([\[link\]](#) and [\[link\]](#)).

An epidemic disease signals the breakdown of an equilibrium in disease frequency, often resulting from some change in environmental conditions or in the population. In the case of influenza, the disruption can be due to antigenic shift or drift (see [Virulence Factors of Bacterial and Viral Pathogens](#)), which allows influenza virus strains to circumvent the acquired immunity of their human hosts.

An epidemic that occurs on a worldwide scale is called a **pandemic disease**. For example, HIV/AIDS is a pandemic disease and novel influenza virus strains often become pandemic.



The 2007–2008 influenza season in the United States saw much higher than normal numbers of visits to emergency departments for influenza-like symptoms as compared to the previous and the following years. (credit: modification of work by Centers for Disease Control and Prevention)



The seasonal epidemic threshold (blue curve) is set by the CDC-based data from the previous five years. When actual mortality rates exceed this threshold, a disease is considered to be epidemic. As this graph shows, pneumonia- and influenza-related mortality saw pronounced epidemics during the winters of 2003–2004, 2005, and 2008. (credit: modification of work by Centers for Disease Control and Prevention)

Note:

- Explain the difference between sporadic and endemic disease.
- Explain the difference between endemic and epidemic disease.

Etiology

When studying an epidemic, an epidemiologist's first task is to determinate the cause of the disease, called the **etiological agent** or **causative agent**. Connecting a disease to a specific pathogen can be challenging because of the extra effort typically required to

demonstrate direct causation as opposed to a simple association. It is not enough to observe an association between a disease and a suspected pathogen; controlled experiments are needed to eliminate other possible causes. In addition, pathogens are typically difficult to detect when there is no immediate clue as to what is causing the outbreak. Signs and symptoms of disease are also commonly nonspecific, meaning that many different agents can give rise to the same set of signs and symptoms. This complicates diagnosis even when a causative agent is familiar to scientists.

Robert Koch was the first scientist to specifically demonstrate the causative agent of a disease (anthrax) in the late 1800s. Koch developed four criteria, now known as Koch's postulates, which had to be met in order to positively link a disease with a pathogenic microbe. Without Koch's postulates, the Golden Age of Microbiology would not have occurred. Between 1876 and 1905, many common diseases were linked with their etiologic agents, including cholera, diphtheria, gonorrhea, meningitis, plague, syphilis, tetanus, and tuberculosis. Today, we use the molecular Koch's postulates, a variation of Koch's original postulates that can be used to establish a link between the disease state and virulence traits unique to a pathogenic strain of a microbe. Koch's original postulates and molecular Koch's postulates were described in more detail in [How Pathogens Cause Disease](#).

Note:

- List some challenges to determining the causative agent of a disease outbreak.

The Role of Public Health Organizations

The main national public health agency in the United States is the **Centers for Disease Control and Prevention (CDC)**, an agency of the Department of Health and Human Services. The CDC is charged with protecting the public from disease and injury. One way that the CDC carries out this mission is by overseeing the National Notifiable Disease Surveillance System (NNDSS) in cooperation with regional, state, and territorial public health departments. The NNDSS monitors diseases considered to be of public health importance on a national scale. Such diseases are called **notifiable diseases or reportable diseases** because all cases must be reported to the CDC. A physician treating a patient with a notifiable disease is legally required to submit a report on the case. Notifiable diseases include HIV infection, measles, West Nile virus infections, and many others. Some states have their own lists of notifiable diseases that include diseases beyond those on the CDC's list.

Notifiable diseases are tracked by epidemiological studies and the data is used to inform health-care providers and the public about possible risks. The CDC publishes the ***Morbidity and Mortality Weekly Report (MMWR)***, which provides physicians and health-care workers with updates on public health issues and the latest data pertaining to notifiable diseases. [\[link\]](#) is an example of the kind of data contained in the *MMWR*.

Incidence of Four Notifiable Diseases in the United States, Week Ending January 2, 2016

Disease	Current Week (Jan 2, 2016)	Median of Previous 52 Weeks	Maximum of Previous 52 Weeks	Cumulative Cases 2015
Campylobacteriosis	406	869	1,385	46,618
<i>Chlamydia trachomatis</i> infection	11,024	28,562	31,089	1,425,303
Giardiasis	115	230	335	11,870
Gonorrhea	3,207	7,155	8,283	369,926

Note:



The current [Morbidity and Mortality Weekly Report](#) is available online.

Note:

- Describe how health agencies obtain data about the incidence of diseases of public health importance.

Key Concepts and Summary

- **Epidemiology** is the science underlying public health.
- **Morbidity** means being in a state of illness, whereas **mortality** refers to death; both **morbidity rates** and **mortality rates** are of interest to epidemiologists.
- **Incidence** is the number of new cases (morbidity or mortality), usually expressed as a proportion, during a specified time period; **prevalence** is the total number affected in the population, again usually expressed as a proportion.
- **Sporadic diseases** only occur rarely and largely without a geographic focus. **Endemic diseases** occur at a constant (and often low) level within a population. **Epidemic diseases** and **pandemic diseases** occur when an outbreak occurs on a significantly larger than expected level, either locally or globally, respectively.
- **Koch's postulates** specify the procedure for confirming a particular pathogen as the etiologic agent of a particular disease. Koch's postulates have limitations in application if the microbe cannot be isolated and cultured or if there is no animal host for the microbe. In this case, molecular Koch's postulates would be utilized.
- In the United States, the **Centers for Disease Control and Prevention** monitors **notifiable diseases** and publishes weekly updates in the *Morbidity and Mortality Weekly Report*.

Critical Thinking

Exercise:**Problem:**

Why might an epidemiological population in a state not be the same size as the number of people in a state? Use an example.

LEARNING OBJECTIVES

- Explain the research approaches used by the pioneers of epidemiology
- Explain how descriptive, analytical, and experimental epidemiological studies go about determining the cause of morbidity and mortality

Epidemiology has its roots in the work of physicians who looked for patterns in disease occurrence as a way to understand how to prevent it. The idea that disease could be transmitted was an important precursor to making sense of some of the patterns. In 1546, Girolamo Fracastoro first proposed the germ theory of disease in his essay *De Contagione et Contagiosis Morbis*, but this theory remained in competition with other theories, such as the miasma hypothesis, for many years (see [What Our Ancestors Knew](#)). Uncertainty about the cause of disease was not an absolute barrier to obtaining useful knowledge from patterns of disease. Some important researchers, such as Florence Nightingale, subscribed to the miasma hypothesis. The transition to acceptance of the germ theory during the 19th century provided a solid mechanistic grounding to the study of disease patterns. The studies of 19th century physicians and researchers such as John Snow, Florence Nightingale, Ignaz Semmelweis, Joseph Lister, Robert Koch, Louis Pasteur, and others sowed the seeds of modern epidemiology.

Pioneers of Epidemiology

John Snow ([\[link\]](#)) was a British physician known as the father of epidemiology for determining the source of the 1854 Broad Street cholera epidemic in London. Based on observations he had made during an earlier cholera outbreak (1848–1849), Snow proposed that cholera was spread through a fecal-oral route of transmission and that a microbe was the infectious agent. He investigated the 1854 cholera epidemic in two ways. First, suspecting that contaminated water was the source of the epidemic, Snow identified the source of water for those infected. He found a high frequency of cholera cases among individuals who obtained their water from the River Thames downstream from London. This water contained the refuse and sewage from London and settlements upstream. He also noted that brewery workers did not contract cholera and on investigation found the owners provided the workers with beer to drink and stated that they likely did not drink water.[\[footnote\]](#) Second, he also painstakingly mapped the incidence of cholera and found a high frequency among those individuals using a particular water pump located on Broad Street. In response to Snow's advice, local officials removed the pump's handle, [\[footnote\]](#) resulting in the containment of the Broad Street cholera epidemic.

John Snow. *On the Mode of Communication of Cholera. Second edition, Much Enlarged*. John Churchill, 1855.

John Snow. "The Cholera near Golden-Wquare, and at Deptford." *Medical Times and Gazette* 9 (1854): 321–322.

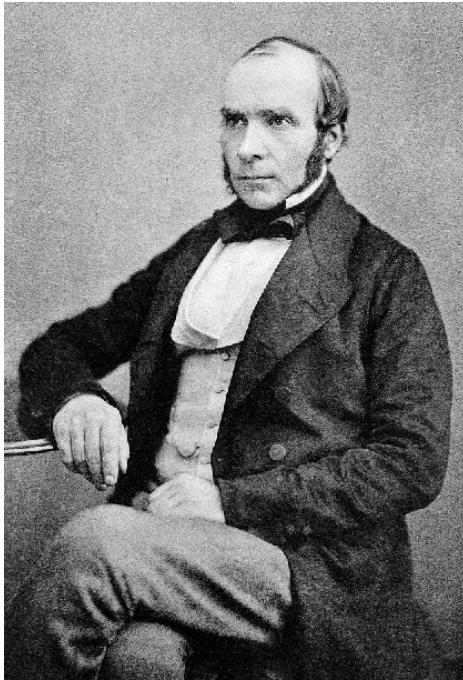
<http://www.ph.ucla.edu/epi/snow/choleragoldensquare.html>.

Snow's work represents an early epidemiological study and it resulted in the first known public health response to an epidemic. Snow's meticulous case-tracking methods are now common practice in studying disease outbreaks and in associating new diseases with their causes. His work further shed light on unsanitary sewage practices and the effects of waste dumping in the Thames. Additionally, his work supported the germ theory of disease, which argued disease could be transmitted through contaminated items, including water contaminated with fecal matter.

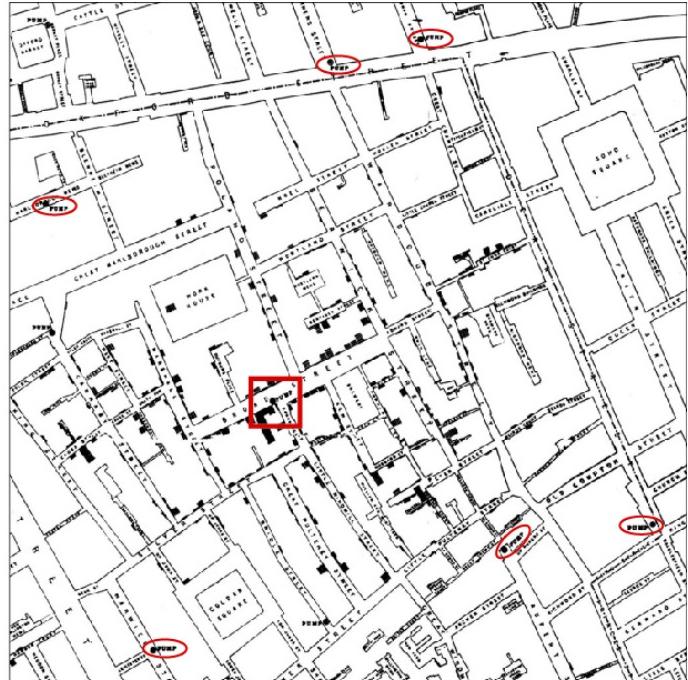
Snow's work illustrated what is referred to today as a **common source spread** of infectious disease, in which there is a single source for all of the individuals infected. In this case, the single source was the contaminated

well below the Broad Street pump. Types of common source spread include point source spread, continuous common source spread, and intermittent common source spread. In **point source spread** of infectious disease, the common source operates for a short time period—less than the incubation period of the pathogen. An example of point source spread is a single contaminated potato salad at a group picnic. In **continuous common source spread**, the infection occurs for an extended period of time, longer than the incubation period. An example of continuous common source spread would be the source of London water taken downstream of the city, which was continuously contaminated with sewage from upstream. Finally, with **intermittent common source spread**, infections occur for a period, stop, and then begin again. This might be seen in infections from a well that was contaminated only after large rainfalls and that cleared itself of contamination after a short period.

In contrast to common source spread, **propagated spread** occurs through direct or indirect person-to-person contact. With propagated spread, there is no single source for infection; each infected individual becomes a source for one or more subsequent infections. With propagated spread, unless the spread is stopped immediately, infections occur for longer than the incubation period. Although point sources often lead to large-scale but localized outbreaks of short duration, propagated spread typically results in longer duration outbreaks that can vary from small to large, depending on the population and the disease ([\[link\]](#)). In addition, because of person-to-person transmission, propagated spread cannot be easily stopped at a single source like point source spread.

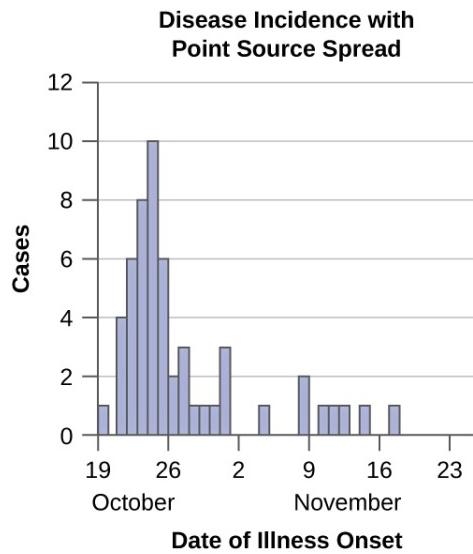


(a)

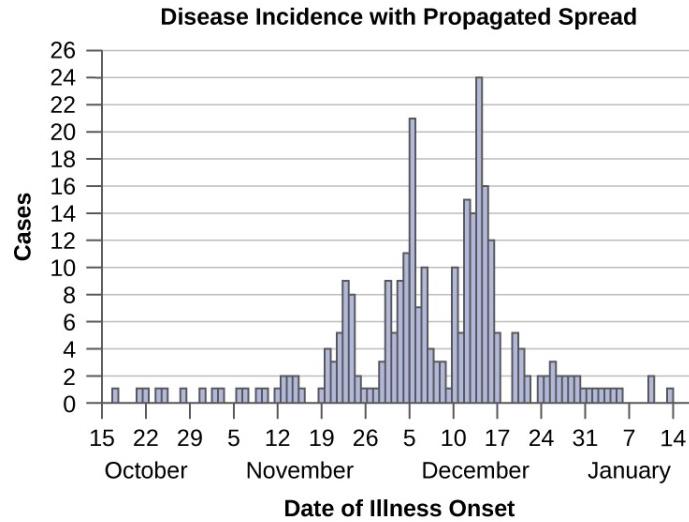


(b)

(a) John Snow (1813–1858), British physician and father of epidemiology. (b) Snow's detailed mapping of cholera incidence led to the discovery of the contaminated water pump on Broad street (red square) responsible for the 1854 cholera epidemic. (credit a: modification of work by “Rsabbatini”/Wikimedia Commons)



(a)



(b)

(a) Outbreaks that can be attributed to point source spread often have a short duration. (b) Outbreaks attributed to propagated spread can have a more extended duration. (credit a, b: modification of work by Centers for Disease Control and Prevention)

Florence Nightingale's work is another example of an early epidemiological study. In 1854, Nightingale was part of a contingent of nurses dispatched by the British military to care for wounded soldiers during the Crimean War. Nightingale kept meticulous records regarding the causes of illness and death during the war. Her recordkeeping was a fundamental task of what would later become the science of epidemiology. Her analysis of the data she collected was published in 1858. In this book, she presented monthly frequency data on causes of death in a wedge chart histogram ([\[link\]](#)). This graphical presentation of data, unusual at the time, powerfully illustrated that the vast majority of casualties during the war occurred not due to wounds sustained in action but to what Nightingale deemed preventable infectious diseases. Often these diseases occurred because of poor sanitation and lack of access to hospital facilities. Nightingale's findings led to many reforms in the British military's system of medical care.

Joseph Lister provided early epidemiological evidence leading to good public health practices in clinics and hospitals. These settings were notorious in the mid-1800s for fatal infections of surgical wounds at a time when the germ theory of disease was not yet widely accepted (see [Foundations of Modern Cell Theory](#)). Most physicians did not wash their hands between patient visits or clean and sterilize their surgical tools. Lister, however, discovered the disinfecting properties of carbolic acid, also known as phenol (see [Using Chemicals to Control Microorganisms](#)). He introduced several disinfection protocols that dramatically lowered post-surgical infection rates. [\[footnote\]](#) He demanded that surgeons who worked for him use a 5% carbolic acid solution to clean their surgical tools between patients, and even went so far as to spray the solution onto bandages and over the surgical site during operations ([\[link\]](#)). He also took precautions not to introduce sources of infection from his skin or clothing by removing his coat, rolling up his sleeves, and washing his hands in a dilute solution of carbolic acid before and during the surgery.

O.M. Lidwell. “Joseph Lister and Infection from the Air.” *Epidemiology and Infection* 99 (1987): 569–578.

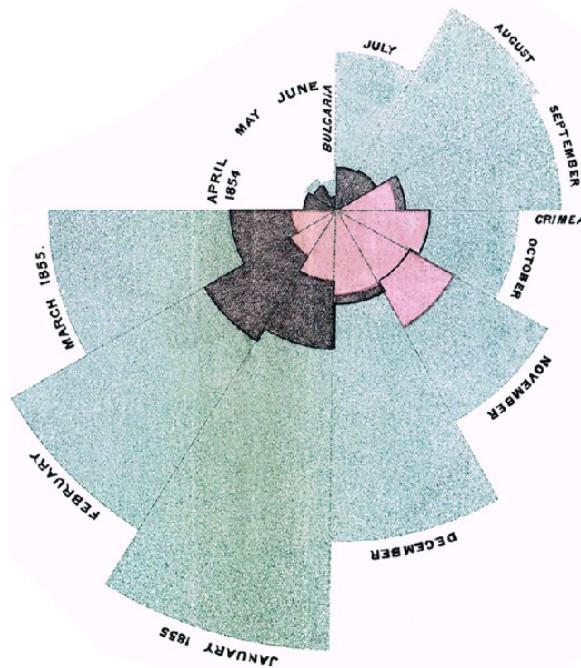
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2249236/pdf/epidinfect00006-0004.pdf>.



(a)

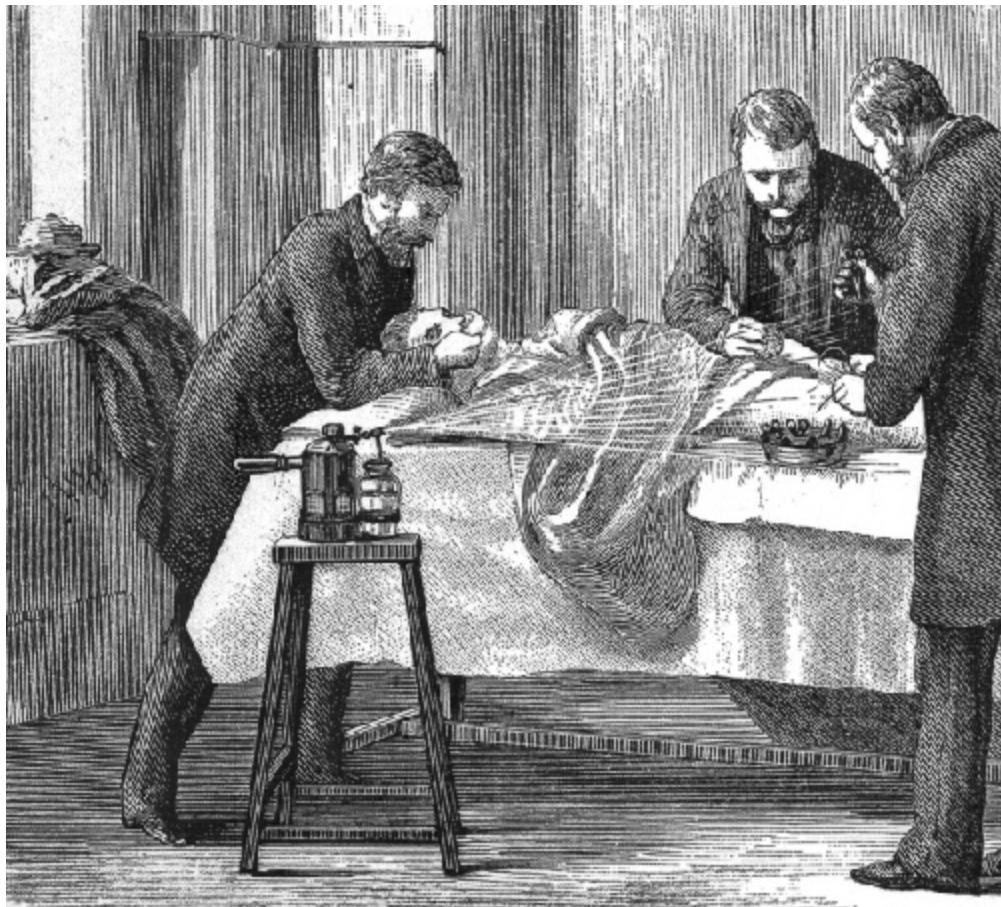
DIAGRAM OF THE CAUSES OF MORTALITY
IN THE ARMY IN THE EAST.

1.
APRIL 1854 TO MARCH 1855.



(b)

(a) Florence Nightingale reported on the data she collected as a nurse in the Crimean War. (b) Nightingale's diagram shows the number of fatalities in soldiers by month of the conflict from various causes. The total number dead in a particular month is equal to the area of the wedge for that month. The colored sections of the wedge represent different causes of death: wounds (pink), preventable infectious diseases (gray), and all other causes (brown).



Joseph Lister initiated the use of a carbolic acid (phenol) during surgeries. This illustration of a surgery shows a pressurized canister of carbolic acid being sprayed over the surgical site.

Note:



Visit the [website](#) for *The Ghost Map*, a book about Snow's work related to the Broad Street pump cholera outbreak.

John Snow's [own account of his work](#) has additional links and information. This [CDC resource](#) further breaks down the pattern expected from a point-source outbreak.

Learn more about [Nightingale's wedge chart](#) here.

Note:

- Explain the difference between common source spread and propagated spread of disease.
- Describe how the observations of John Snow, Florence Nightingale, and Joseph Lister led to improvements in public health.

Types of Epidemiological Studies

Today, epidemiologists make use of study designs, the manner in which data are gathered to test a hypothesis, similar to those of researchers studying other phenomena that occur in populations. These approaches can be divided into observational studies (in which subjects are not manipulated) and experimental studies (in which subjects are manipulated). Collectively, these studies give modern-day epidemiologists multiple tools for exploring the connections between infectious diseases and the populations of susceptible individuals they might infect.

Observational Studies

In an **observational study**, data are gathered from study participants through measurements (such as physiological variables like white blood cell count), or answers to questions in interviews (such as recent travel or exercise frequency). The subjects in an observational study are typically chosen at random from a population of affected or unaffected individuals. However, the subjects in an observational study are in no way manipulated by the researcher. Observational studies are typically easier to carry out than experimental studies, and in certain situations they may be the only studies possible for ethical reasons.

Observational studies are only able to measure associations between disease occurrence and possible causative agents; they do not necessarily prove a causal relationship. For example, suppose a study finds an association between heavy coffee drinking and lower incidence of skin cancer. This might suggest that coffee prevents skin cancer, but there may be another unmeasured factor involved, such as the amount of sun exposure the participants receive. If it turns out that coffee drinkers work more in offices and spend less time outside in the sun than those who drink less coffee, then it may be possible that the lower rate of skin cancer is due to less sun exposure, not to coffee consumption. The observational study cannot distinguish between these two potential causes.

There are several useful approaches in observational studies. These include methods classified as descriptive epidemiology and analytical epidemiology. **Descriptive epidemiology** gathers information about a disease outbreak, the affected individuals, and how the disease has spread over time in an exploratory stage of study. This type of study will involve interviews with patients, their contacts, and their family members; examination of samples and medical records; and even histories of food and beverages consumed. Such a study might be conducted while the outbreak is still occurring. Descriptive studies might form the basis for developing a hypothesis of causation that could be tested by more rigorous observational and experimental studies.

Analytical epidemiology employs carefully selected groups of individuals in an attempt to more convincingly evaluate hypotheses about potential

causes for a disease outbreak. The selection of cases is generally made at random, so the results are not biased because of some common characteristic of the study participants. Analytical studies may gather their data by going back in time (retrospective studies), or as events unfold forward in time (prospective studies).

Retrospective studies gather data from the past on present-day cases. Data can include things like the medical history, age, gender, or occupational history of the affected individuals. This type of study examines associations between factors chosen or available to the researcher and disease occurrence.

Prospective studies follow individuals and monitor their disease state during the course of the study. Data on the characteristics of the study subjects and their environments are gathered at the beginning and during the study so that subjects who become ill may be compared with those who do not. Again, the researchers can look for associations between the disease state and variables that were measured during the study to shed light on possible causes.

Analytical studies incorporate groups into their designs to assist in teasing out associations with disease. Approaches to group-based analytical studies include cohort studies, case-control studies, and cross-sectional studies. The **cohort method** examines groups of individuals (called cohorts) who share a particular characteristic. For example, a cohort might consist of individuals born in the same year and the same place; or it might consist of people who practice or avoid a particular behavior, e.g., smokers or nonsmokers. In a cohort study, cohorts can be followed prospectively or studied retrospectively. If only a single cohort is followed, then the affected individuals are compared with the unaffected individuals in the same group. Disease outcomes are recorded and analyzed to try to identify correlations between characteristics of individuals in the cohort and disease incidence. Cohort studies are a useful way to determine the causes of a condition without violating the ethical prohibition of exposing subjects to a risk factor. Cohorts are typically identified and defined based on suspected risk factors to which individuals have already been exposed through their own choices or circumstances.

Case-control studies are typically retrospective and compare a group of individuals with a disease to a similar group of individuals without the disease. Case-control studies are far more efficient than cohort studies because researchers can deliberately select subjects who are already affected with the disease as opposed to waiting to see which subjects from a random sample will develop a disease.

A **cross-sectional study** analyzes randomly selected individuals in a population and compares individuals affected by a disease or condition to those unaffected at a single point in time. Subjects are compared to look for associations between certain measurable variables and the disease or condition. Cross-sectional studies are also used to determine the prevalence of a condition.

Experimental Studies

Experimental epidemiology uses laboratory or clinical studies in which the investigator manipulates the study subjects to study the connections between diseases and potential causative agents or to assess treatments. Examples of treatments might be the administration of a drug, the inclusion or exclusion of different dietary items, physical exercise, or a particular surgical procedure. Animals or humans are used as test subjects. Because **experimental studies** involve manipulation of subjects, they are typically more difficult and sometimes impossible for ethical reasons.

Koch's postulates require experimental interventions to determine the causative agent for a disease. Unlike observational studies, experimental studies can provide strong evidence supporting cause because other factors are typically held constant when the researcher manipulates the subject. The outcomes for one group receiving the treatment are compared to outcomes for a group that does not receive the treatment but is treated the same in every other way. For example, one group might receive a regimen of a drug administered as a pill, while the untreated group receives a placebo (a pill that looks the same but has no active ingredient). Both groups are treated as similarly as possible except for the administration of the drug. Because other variables are held constant in both the treated and the untreated

groups, the researcher is more certain that any change in the treated group is a result of the specific manipulation.

Experimental studies provide the strongest evidence for the etiology of disease, but they must also be designed carefully to eliminate subtle effects of bias. Typically, experimental studies with humans are conducted as double-blind studies, meaning neither the subjects nor the researchers know who is a treatment case and who is not. This design removes a well-known cause of bias in research called the placebo effect, in which knowledge of the treatment by either the subject or the researcher can influence the outcomes.

Note:

- Describe the advantages and disadvantages of observational studies and experimental studies.
- Explain the ways that groups of subjects can be selected for analytical studies.

Key Concepts and Summary

- Early pioneers of epidemiology such as John Snow, Florence Nightingale, and Joseph Lister, studied disease at the population level and used data to disrupt disease transmission.
- **Descriptive epidemiology** studies rely on case analysis and patient histories to gain information about outbreaks, frequently while they are still occurring.
- **Retrospective epidemiology** studies use historical data to identify associations with the disease state of present cases. **Prospective epidemiology** studies gather data and follow cases to find associations with future disease states.
- **Analytical epidemiology** studies are observational studies that are carefully designed to compare groups and uncover associations

between environmental or genetic factors and disease.

- **Experimental epidemiology** studies generate strong evidence of causation in disease or treatment by manipulating subjects and comparing them with control subjects.

Short Answer

Exercise:

Problem:

What activity did John Snow conduct, other than mapping, that contemporary epidemiologists also use when trying to understand how to control a disease?

Modes of Disease Transmission

LEARNING OBJECTIVES

- Describe the different types of disease reservoirs
- Define passive, active, and asymptomatic carrier
- Compare contact, vector, and vehicle modes of transmission
- Identify important disease vectors
- Explain the prevalence of nosocomial infections

Understanding how infectious pathogens spread is critical to preventing infectious disease. Many pathogens require a living host to survive, while others may be able to persist in a dormant state outside of a living host. But having infected one host, all pathogens must also have a mechanism of transfer from one host to another or they will die when their host dies. Pathogens often have elaborate adaptations to exploit host biology, behavior, and ecology to live in and move between hosts. Hosts have evolved defenses against pathogens, but because their rates of evolution are typically slower than their pathogens (because their generation times are longer), hosts are usually at an evolutionary disadvantage. This section will explore where pathogens survive—both inside and outside hosts—and some of the many ways they move from one host to another.

Reservoirs and Carriers

For pathogens to persist over long periods of time they require **reservoirs** where they normally reside. Reservoirs can be living organisms or nonliving sites. Nonliving reservoirs can include soil and water in the environment. These may naturally harbor the organism because it may grow

in that environment. These environments may also become contaminated with pathogens in human feces, pathogens shed by intermediate hosts, or pathogens contained in the remains of intermediate hosts.

Pathogens may have mechanisms of dormancy or resilience that allow them to survive (but typically not to reproduce) for varying periods of time in nonliving environments. For example, *Clostridium tetani* survives in the soil and in the presence of oxygen as a resistant endospore. Although many viruses are soon destroyed once in contact with air, water, or other non-physiological conditions, certain types are capable of persisting outside of a living cell for varying amounts of time. For example, a study that looked at the ability of influenza viruses to infect a cell culture after varying amounts of time on a banknote showed survival times from 48 hours to 17 days, depending on how they were deposited on the banknote.[\[footnote\]](#) On the other hand, cold-causing rhinoviruses are somewhat fragile, typically surviving less than a day outside of physiological fluids.

Yves Thomas, Guido Vogel, Werner Wunderli, Patricia Suter, Mark Witschi, Daniel Koch, Caroline Tapparel, and Laurent Kaiser. “Survival of Influenza Virus on Banknotes.” *Applied and Environmental Microbiology* 74, no. 10 (2008): 3002–3007.

A human acting as a reservoir of a pathogen may or may not be capable of transmitting the pathogen, depending on the stage of infection and the pathogen. To help prevent the spread of disease among school children, the CDC has developed guidelines based on the risk of transmission during the course of the disease. For example, children with chickenpox are considered contagious for five days from the start of the rash, whereas children with most gastrointestinal illnesses should be kept home for 24 hours after the symptoms disappear.

An individual capable of transmitting a pathogen without displaying symptoms is referred to as a carrier. A **passive carrier** is contaminated with the pathogen and can mechanically transmit it to another host; however, a passive carrier is not infected. For example, a health-care professional who fails to wash his hands after seeing a patient harboring an infectious agent could become a passive carrier, transmitting the pathogen to another patient who becomes infected.

By contrast, an **active carrier** is an infected individual who can transmit the disease to others. An active carrier may or may not exhibit signs or symptoms of infection. For example, active carriers may transmit the disease during the incubation period (before they show signs and symptoms) or the period of convalescence (after symptoms have subsided). Active carriers who do not present signs or symptoms of disease despite infection are called **asymptomatic carriers**. Pathogens such as hepatitis B virus, herpes simplex virus, and HIV are frequently transmitted by asymptomatic carriers. Mary Mallon, better known as Typhoid Mary, is a famous historical example of an asymptomatic carrier. An Irish immigrant, Mallon worked as a cook for households in and around New York City between 1900 and 1915. In each household, the residents developed typhoid fever (caused by *Salmonella typhi*) a few weeks after Mallon started working. Later investigations determined that Mallon was responsible for at least 122 cases of typhoid fever, five of which were fatal.[\[footnote\]](#) See [Eye on Ethics: Typhoid Mary](#) for more about the Mallon case.

Filio Marineli, Gregory Tsoucalas, Marianna Karamanou, and George Androutsos. “Mary Mallon (1869–1938) and the History of Typhoid Fever.” *Annals of Gastroenterology* 26 (2013): 132–134.
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3959940/pdf/AnnGastroenterol-26-132.pdf>.

A pathogen may have more than one living reservoir. In zoonotic diseases, animals act as reservoirs of human disease and transmit the infectious agent to humans through direct or indirect contact. In some cases, the disease also affects the animal, but in other cases the animal is asymptomatic.

In parasitic infections, the parasite’s preferred host is called the **definitive host**. In parasites with complex life cycles, the definitive host is the host in which the parasite reaches sexual maturity. Some parasites may also infect one or more **intermediate hosts** in which the parasite goes through several immature life cycle stages or reproduces asexually.

Note:



George Soper, the sanitary engineer who traced the typhoid outbreak to Mary Mallon, [gives an account](#) of his investigation, an example of descriptive epidemiology, in “The Curious Career of Typhoid Mary.”

Note:

- List some nonliving reservoirs for pathogens.
- Explain the difference between a passive carrier and an active carrier.

Transmission

Regardless of the reservoir, transmission must occur for an infection to spread. First, transmission from the reservoir to the individual must occur. Then, the individual must transmit the infectious agent to other susceptible individuals, either directly or indirectly. Pathogenic microorganisms employ diverse transmission mechanisms.

Contact Transmission

Contact transmission includes direct contact or indirect contact. Person-to-person transmission is a form of **direct contact transmission**. Here the agent is transmitted by physical contact between two individuals ([\[link\]](#)) through actions such as touching, kissing, sexual intercourse, or droplet

sprays. Direct contact can be categorized as vertical, horizontal, or droplet transmission. **Vertical direct contact transmission** occurs when pathogens are transmitted from mother to child during pregnancy, birth, or breastfeeding. Other kinds of direct contact transmission are called **horizontal direct contact transmission**. Often, contact between mucous membranes is required for entry of the pathogen into the new host, although skin-to-skin contact can lead to mucous membrane contact if the new host subsequently touches a mucous membrane. Contact transmission may also be site-specific; for example, some diseases can be transmitted by sexual contact but not by other forms of contact.

When an individual coughs or sneezes, small droplets of mucus that may contain pathogens are ejected. This leads to direct **droplet transmission**, which refers to droplet transmission of a pathogen to a new host over distances of one meter or less. A wide variety of diseases are transmitted by droplets, including influenza and many forms of pneumonia. Transmission over distances greater than one meter is called airborne transmission.

Indirect contact transmission involves inanimate objects called fomites that become contaminated by pathogens from an infected individual or reservoir ([\[link\]](#)). For example, an individual with the common cold may sneeze, causing droplets to land on a fomite such as a tablecloth or carpet, or the individual may wipe her nose and then transfer mucus to a fomite such as a doorknob or towel. Transmission occurs indirectly when a new susceptible host later touches the fomite and transfers the contaminated material to a susceptible portal of entry. Fomites can also include objects used in clinical settings that are not properly sterilized, such as syringes, needles, catheters, and surgical equipment. Pathogens transmitted indirectly via such fomites are a major cause of healthcare-associated infections (see [Controlling Microbial Growth](#)).



Direct contact transmission of pathogens can occur through physical contact. Many pathogens require contact with a mucous membrane to enter the body, but the host may transfer the pathogen from another point of contact (e.g., hand) to a mucous membrane (e.g., mouth or eye). (credit left: modification of work by Lisa Doehnert)



Fomites are nonliving objects that facilitate the indirect transmission of pathogens. Contaminated doorknobs, towels, and syringes are all common examples of fomites. (credit left: modification of work by Kate Ter Haar; credit middle: modification of work by Vernon Swanepoel; credit right: modification of work by “Zaldylmg”/Flickr)

Vehicle Transmission

The term **vehicle transmission** refers to the transmission of pathogens through vehicles such as water, food, and air. Water contamination through poor sanitation methods leads to waterborne transmission of disease.

Waterborne disease remains a serious problem in many regions throughout the world. The World Health Organization (WHO) estimates that contaminated drinking water is responsible for more than 500,000 deaths each year.[\[footnote\]](#) Similarly, food contaminated through poor handling or storage can lead to foodborne transmission of disease ([\[link\]](#)).

World Health Organization. Fact sheet No. 391—*Drinking Water*. June 2005. <http://www.who.int/mediacentre/factsheets/fs391/en>.

Dust and fine particles known as aerosols, which can float in the air, can carry pathogens and facilitate the airborne transmission of disease. For example, dust particles are the dominant mode of transmission of hantavirus to humans. Hantavirus is found in mouse feces, urine, and saliva, but when these substances dry, they can disintegrate into fine particles that can become airborne when disturbed; inhalation of these particles can lead to a serious and sometimes fatal respiratory infection.

Although droplet transmission over short distances is considered contact transmission as discussed above, longer distance transmission of droplets through the air is considered vehicle transmission. Unlike larger particles that drop quickly out of the air column, fine mucus droplets produced by coughs or sneezes can remain suspended for long periods of time, traveling considerable distances. In certain conditions, droplets desiccate quickly to produce a droplet nucleus that is capable of transmitting pathogens; air temperature and humidity can have an impact on effectiveness of airborne transmission.

Tuberculosis is often transmitted via airborne transmission when the causative agent, *Mycobacterium tuberculosis*, is released in small particles with coughs. Because tuberculosis requires as few as 10 microbes to initiate a new infection, patients with tuberculosis must be treated in rooms equipped with special ventilation, and anyone entering the room should wear a mask.



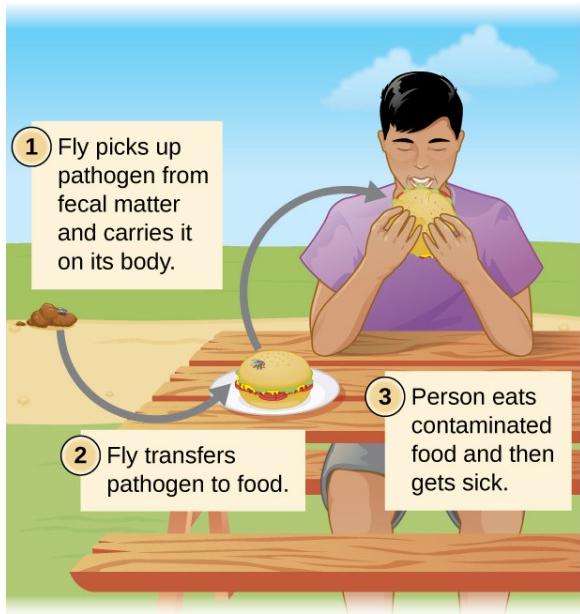
Food is an important vehicle of transmission for pathogens, especially of the gastrointestinal and upper respiratory systems. Notice the glass shield above the food trays, designed to prevent pathogens ejected in coughs and sneezes from entering the food. (credit: Fort George G. Meade Public Affairs Office)

Vector Transmission

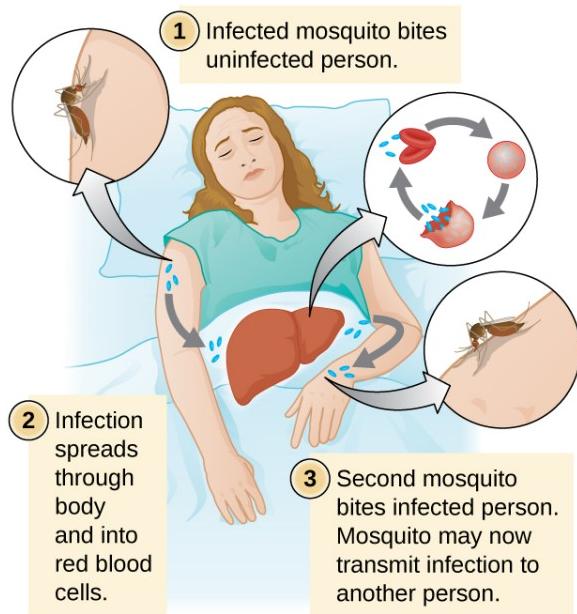
Diseases can also be transmitted by a mechanical or biological vector, an animal (typically an arthropod) that carries the disease from one host to another. **Mechanical transmission** is facilitated by a **mechanical vector**, an animal that carries a pathogen from one host to another without being infected itself. For example, a fly may land on fecal matter and later transmit bacteria from the feces to food that it lands on; a human eating the food may then become infected by the bacteria, resulting in a case of diarrhea or dysentery ([\[link\]](#)).

Biological transmission occurs when the pathogen reproduces within a **biological vector** that transmits the pathogen from one host to another ([\[link\]](#)). Arthropods are the main vectors responsible for biological transmission ([\[link\]](#)). Most arthropod vectors transmit the pathogen by biting the host, creating a wound that serves as a portal of entry. The pathogen may go through part of its reproductive cycle in the gut or salivary glands of the arthropod to facilitate its transmission through the bite. For example, hemipterans (called “kissing bugs” or “assassin bugs”) transmit Chagas disease to humans by defecating when they bite, after which the human scratches or rubs the infected feces into a mucous membrane or break in the skin.

Biological insect vectors include mosquitoes, which transmit malaria and other diseases, and lice, which transmit typhus. Other arthropod vectors can include arachnids, primarily ticks, which transmit Lyme disease and other diseases, and mites, which transmit scrub typhus and rickettsial pox. Biological transmission, because it involves survival and reproduction within a parasitized vector, complicates the biology of the pathogen and its transmission. There are also important non-arthropod vectors of disease, including mammals and birds. Various species of mammals can transmit rabies to humans, usually by means of a bite that transmits the rabies virus. Chickens and other domestic poultry can transmit avian influenza to humans through direct or indirect contact with avian influenza virus A shed in the birds’ saliva, mucous, and feces.



(a)



(b)

(a) A mechanical vector carries a pathogen on its body from one host to another, not as an infection. (b) A biological vector carries a pathogen from one host to another after becoming infected itself.

Common Arthropod Vectors and Select Pathogens			
Vector	Species	Pathogen	Disease
Black fly	<i>Simulium</i> spp.	<i>Onchocerca volvulus</i>	Onchocerciasis (river blindness)
Flea	<i>Xenopsylla cheopis</i>	<i>Rickettsia typhi</i>	Murine typhus
		<i>Yersinia pestis</i>	Plague
Kissing bug	<i>Triatoma</i> spp.	<i>Trypanosoma cruzi</i>	Chagas disease
Louse	<i>Pediculus humanus humanus</i>	<i>Bartonella quintana</i>	Trench fever
		<i>Borrelia recurrentis</i>	Relapsing fever
		<i>Rickettsia prowazekii</i>	Typhus
Mite (chigger)	<i>Leptotrombidium</i> spp.	<i>Orientia tsutsugamushi</i>	Scrub typhus
	<i>Liponyssoides sanguineus</i>	<i>Rickettsia akari</i>	Rickettsialpox
Mosquito	<i>Aedes</i> spp., <i>Haemagogus</i> spp.	<i>Yellow fever virus</i>	Yellow fever
	<i>Anopheles</i> spp.	<i>Plasmodium falciparum</i>	Malaria
	<i>Culex pipiens</i>	<i>West Nile virus</i>	West Nile disease
Sand fly	<i>Phlebotomus</i> spp.	<i>Leishmania</i> spp.	Leishmaniasis
Tick	<i>Ixodes</i> spp.	<i>Borrelia</i> spp.	Lyme disease
	<i>Dermacentor</i> spp. and others	<i>Rickettsia rickettsii</i>	Rocky Mountain spotted fever
Tsetse fly	<i>Glossina</i> spp.	<i>Trypanosoma brucei</i>	African trypanosomiasis (sleeping sickness)

(credit “Black fly”, “Tick”, “Tsetse fly”: modification of work by USDA; credit: “Flea”: modification of work by Centers for Disease Control and Prevention; credit: “Louse”, “Mosquito”, “Sand fly”:

modification of work by James Gathany, Centers for Disease Control and Prevention; credit “Kissing bug”: modification of work by Glenn Seplak; credit “Mite”: modification of work by Michael Wunderli)

Note:

- Describe how diseases can be transmitted through the air.
- Explain the difference between a mechanical vector and a biological vector.

Note:

Using GMOs to Stop the Spread of Zika

In 2016, an epidemic of the Zika virus was linked to a high incidence of birth defects in South America and Central America. As winter turned to spring in the northern hemisphere, health officials correctly predicted the virus would spread to North America, coinciding with the breeding season of its major vector, the *Aedes aegypti* mosquito.

The range of the *A. aegypti* mosquito extends well into the southern United States ([\[link\]](#)). Because these same mosquitoes serve as vectors for other problematic diseases (dengue fever, yellow fever, and others), various methods of mosquito control have been proposed as solutions. Chemical pesticides have been used effectively in the past, and are likely to be used again; but because chemical pesticides can have negative impacts on the environment, some scientists have proposed an alternative that involves genetically engineering *A. aegypti* so that it cannot reproduce. This method, however, has been the subject of some controversy.

One method that has worked in the past to control pests, with little apparent downside, has been sterile male introductions. This method controlled the screw-worm fly pest in the southwest United States and fruit fly pests of fruit crops. In this method, males of the target species are reared in the lab, sterilized with radiation, and released into the

environment where they mate with wild females, who subsequently bear no live offspring. Repeated releases shrink the pest population.

A similar method, taking advantage of recombinant DNA technology, [footnote] introduces a dominant lethal allele into male mosquitoes that is suppressed in the presence of tetracycline (an antibiotic) during laboratory rearing. The males are released into the environment and mate with female mosquitoes. Unlike the sterile male method, these matings produce offspring, but they die as larvae from the lethal gene in the absence of tetracycline in the environment. As of 2016, this method has yet to be implemented in the United States, but a UK company tested the method in Piracicaba, Brazil, and found an 82% reduction in wild *A. aegypti* larvae and a 91% reduction in dengue cases in the treated area.[footnote] In August 2016, amid news of Zika infections in several Florida communities, the FDA gave the UK company permission to test this same mosquito control method in Key West, Florida, pending compliance with local and state regulations and a referendum in the affected communities.

Blandine Massonnet-Bruneel, Nicole Corre-Catelin, Renaud Lacroix, Rosemary S. Lees, Kim Phuc Hoang, Derric Nimmo, Luke Alphey, and Paul Reiter. “Fitness of Transgenic Mosquito *Aedes aegypti* Males Carrying a Dominant Lethal Genetic System.” *PLOS ONE* 8, no. 5 (2013): e62711.

Richard Levine. “Cases of Dengue Drop 91 Percent Due to Genetically Modified Mosquitoes.” *Entomology Today*.
<https://entomologytoday.org/2016/07/14/cases-of-dengue-drop-91-due-to-genetically-modified-mosquitoes>.

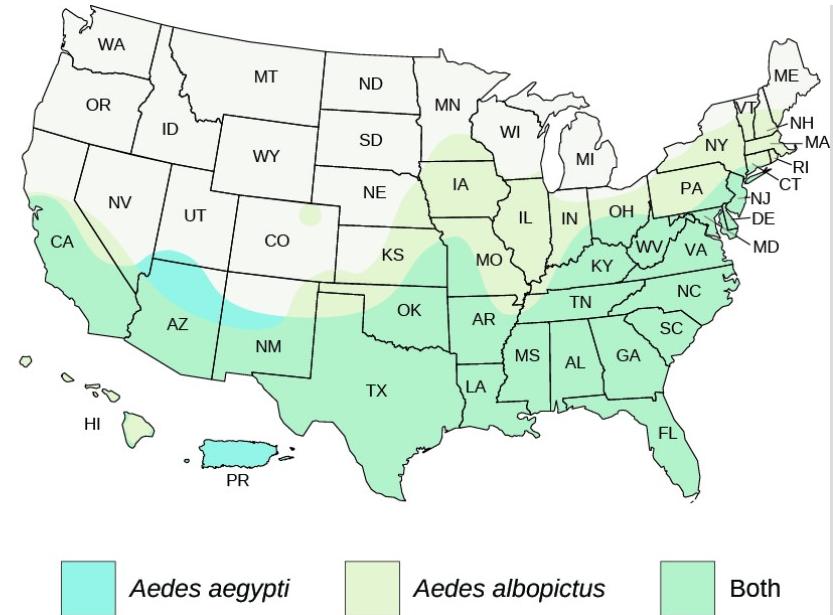
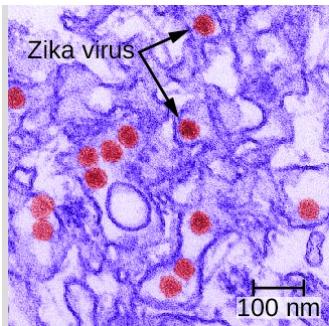
The use of genetically modified organisms (GMOs) to control a disease vector has its advocates as well as its opponents. In theory, the system could be used to drive the *A. aegypti* mosquito extinct—a noble goal according to some, given the damage they do to human populations. [footnote] But opponents of the idea are concerned that the gene could escape the species boundary of *A. aegypti* and cause problems in other species, leading to unforeseen ecological consequences. Opponents are also wary of the program because it is being administered by a for-profit corporation, creating the potential for conflicts of interest that would have to be tightly regulated; and it is not clear how any unintended consequences of the program could be reversed.

Olivia Judson. “A Bug’s Death.” *The New York Times*, September 25, 2003. <http://www.nytimes.com/2003/09/25/opinion/a-bug-s-death.html>. There are other epidemiological considerations as well. *Aedes aegypti* is apparently not the only vector for the Zika virus. *Aedes albopictus*, the Asian tiger mosquito, is also a vector for the Zika virus.[\[footnote\]](#) *A. albopictus* is now widespread around the planet including much of the United States ([\[link\]](#)). Many other mosquitoes have been found to harbor Zika virus, though their capacity to act as vectors is unknown.[\[footnote\]](#) Genetically modified strains of *A. aegypti* will not control the other species of vectors. Finally, the Zika virus can apparently be transmitted sexually between human hosts, from mother to child, and possibly through blood transfusion. All of these factors must be considered in any approach to controlling the spread of the virus.

Gilda Grard, Mélanie Caron, Illich Manfred Mombo, Dieudonné Nkoghe, Statiana Mboui Ondo, Davy Jiolle, Didier Fontenille, Christophe Paupy, and Eric Maurice Leroy. “Zika Virus in Gabon (Central Africa)—2007: A New Threat from *Aedes albopictus*?” *PLOS Neglected Tropical Diseases* 8, no. 2 (2014): e2681.

Constância F.J. Ayres. “Identification of Zika Virus Vectors and Implications for Control.” *The Lancet Infectious Diseases* 16, no. 3 (2016): 278–279.

Clearly there are risks and unknowns involved in conducting an open-environment experiment of an as-yet poorly understood technology. But allowing the Zika virus to spread unchecked is also risky. Does the threat of a Zika epidemic justify the ecological risk of genetically engineering mosquitos? Are current methods of mosquito control sufficiently ineffective or harmful that we need to try untested alternatives? These are the questions being put to public health officials now.



The Zika virus is an enveloped virus transmitted by mosquitoes, especially *Aedes aegypti*. The range of this mosquito includes much of the United States, from the Southwest and Southeast to as far north as the Mid-Atlantic. The range of *A. albopictus*, another vector, extends even farther north to New England and parts of the Midwest.

(credit micrograph: modification of work by Cynthia Goldsmith, Centers for Disease Control and Prevention; credit photo: modification of work by James Gathany, Centers for Disease Control and Prevention; credit map: modification of work by Centers for Disease Control and Prevention)

Quarantining

Individuals suspected or known to have been exposed to certain contagious pathogens may be **quarantined**, or isolated to prevent transmission of the disease to others. Hospitals and other health-care facilities generally set up special wards to isolate patients with particularly hazardous diseases such as tuberculosis or Ebola ([\[link\]](#)). Depending on the setting, these wards may be equipped with special air-handling methods, and personnel may

implement special protocols to limit the risk of transmission, such as personal protective equipment or the use of chemical disinfectant sprays upon entry and exit of medical personnel.

The duration of the quarantine depends on factors such as the incubation period of the disease and the evidence suggestive of an infection. The patient may be released if signs and symptoms fail to materialize when expected or if preventive treatment can be administered in order to limit the risk of transmission. If the infection is confirmed, the patient may be compelled to remain in isolation until the disease is no longer considered contagious.

In the United States, public health authorities may only quarantine patients for certain diseases, such as cholera, diphtheria, infectious tuberculosis, and strains of influenza capable of causing a pandemic. Individuals entering the United States or moving between states may be quarantined by the CDC if they are suspected of having been exposed to one of these diseases.

Although the CDC routinely monitors entry points to the United States for crew or passengers displaying illness, quarantine is rarely implemented.



(a)



(b)

(a) The Aeromedical Biological Containment System (ABCS) is a module designed by the CDC and Department of Defense specifically for transporting highly contagious patients by air. (b) An isolation ward for Ebola patients in Lagos, Nigeria. (credit a: modification of

work by Centers for Disease Control and Prevention; credit b:
modification of work by CDC Global)

Healthcare-Associated (Nosocomial) Infections

Hospitals, retirement homes, and prisons attract the attention of epidemiologists because these settings are associated with increased incidence of certain diseases. Higher rates of transmission may be caused by characteristics of the environment itself, characteristics of the population, or both. Consequently, special efforts must be taken to limit the risks of infection in these settings.

Infections acquired in health-care facilities, including hospitals, are called **nosocomial infections or healthcare-associated infections (HAI)**. HAIs are often connected with surgery or other invasive procedures that provide the pathogen with access to the portal of infection. For an infection to be classified as an HAI, the patient must have been admitted to the health-care facility for a reason other than the infection. In these settings, patients suffering from primary disease are often afflicted with compromised immunity and are more susceptible to secondary infection and opportunistic pathogens.

In 2011, more than 720,000 HAIs occurred in hospitals in the United States, according to the CDC. About 22% of these HAIs occurred at a surgical site, and cases of pneumonia accounted for another 22%; urinary tract infections accounted for an additional 13%, and primary bloodstream infections 10%. [\[footnote\]](#) Such HAIs often occur when pathogens are introduced to patients' bodies through contaminated surgical or medical equipment, such as catheters and respiratory ventilators. Health-care facilities seek to limit nosocomial infections through training and hygiene protocols such as those described in [Control of Microbial Growth](#).

Centers for Disease Control and Prevention. "HAI Data and Statistics." 2016. <http://www.cdc.gov/hai/surveillance>. Accessed Jan 2, 2016.

Note:

- Give some reasons why HAIs occur.

Key Concepts and Summary

- **Reservoirs** of human disease can include the human and animal populations, soil, water, and inanimate objects or materials.
- **Contact transmission** can be **direct** or **indirect** through physical contact with either an infected host (direct) or contact with a fomite that an infected host has made contact with previously (indirect).
- Vector transmission occurs when a living organism carries an infectious agent on its body (**mechanical**) or as an infection host itself (**biological**), to a new host.
- **Vehicle transmission** occurs when a substance, such as soil, water, or air, carries an infectious agent to a new host.
- **Healthcare-associated infections (HAI)**, or **nosocomial infections**, are acquired in a clinical setting. Transmission is facilitated by medical interventions and the high concentration of susceptible, immunocompromised individuals in clinical settings.

Critical Thinking

Exercise:**Problem:**

Many people find that they become ill with a cold after traveling by airplane. The air circulation systems of commercial aircraft use HEPA filters that should remove any infectious agents that pass through them. What are the possible reasons for increased incidence of colds after flights?

LEARNING OBJECTIVES

- Describe the entities involved in international public health and their activities
- Identify and differentiate between emerging and reemerging infectious diseases

A large number of international programs and agencies are involved in efforts to promote global public health. Among their goals are developing infrastructure in health care, public sanitation, and public health capacity; monitoring infectious disease occurrences around the world; coordinating communications between national public health agencies in various countries; and coordinating international responses to major health crises. In large part, these international efforts are necessary because disease-causing microorganisms know no national boundaries.

The World Health Organization (WHO)

International public health issues are coordinated by the **World Health Organization (WHO)**, an agency of the United Nations. Of its roughly \$4 billion budget for 2015–16 [[footnote](#)], about \$1 billion was funded by member states and the remaining \$3 billion by voluntary contributions. In addition to monitoring and reporting on infectious disease, WHO also develops and implements strategies for their control and prevention. WHO has had a number of successful international public health campaigns. For example, its vaccination program against smallpox, begun in the mid-1960s, resulted in the global eradication of the disease by 1980. WHO continues to be involved in infectious disease control, primarily in the developing world, with programs targeting malaria, HIV/AIDS, and tuberculosis, among others. It also runs programs to reduce illness and mortality that occur as a result of violence, accidents, lifestyle-associated illnesses such as diabetes, and poor health-care infrastructure.

World Health Organization. “Programme Budget 2014–2015.”
<http://www.who.int/about/finances-accountability/budget/en>.

WHO maintains a global alert and response system that coordinates information from member nations. In the event of a public health emergency or epidemic, it provides

logistical support and coordinates international response to the emergency. The United States contributes to this effort through the CDC. The CDC carries out international monitoring and public health efforts, mainly in the service of protecting US public health in an increasingly connected world. Similarly, the European Union maintains a Health Security Committee that monitors disease outbreaks within its member countries and internationally, coordinating with WHO.

Note:

- Name the organizations that participate in international public health monitoring.

Emerging and Reemerging Infectious Diseases

Both WHO and some national public health agencies such as the CDC monitor and prepare for **emerging infectious diseases**. An emerging infectious disease is either new to the human population or has shown an increase in prevalence in the previous twenty years. Whether the disease is new or conditions have changed to cause an increase in frequency, its status as emerging implies the need to apply resources to understand and control its growing impact.

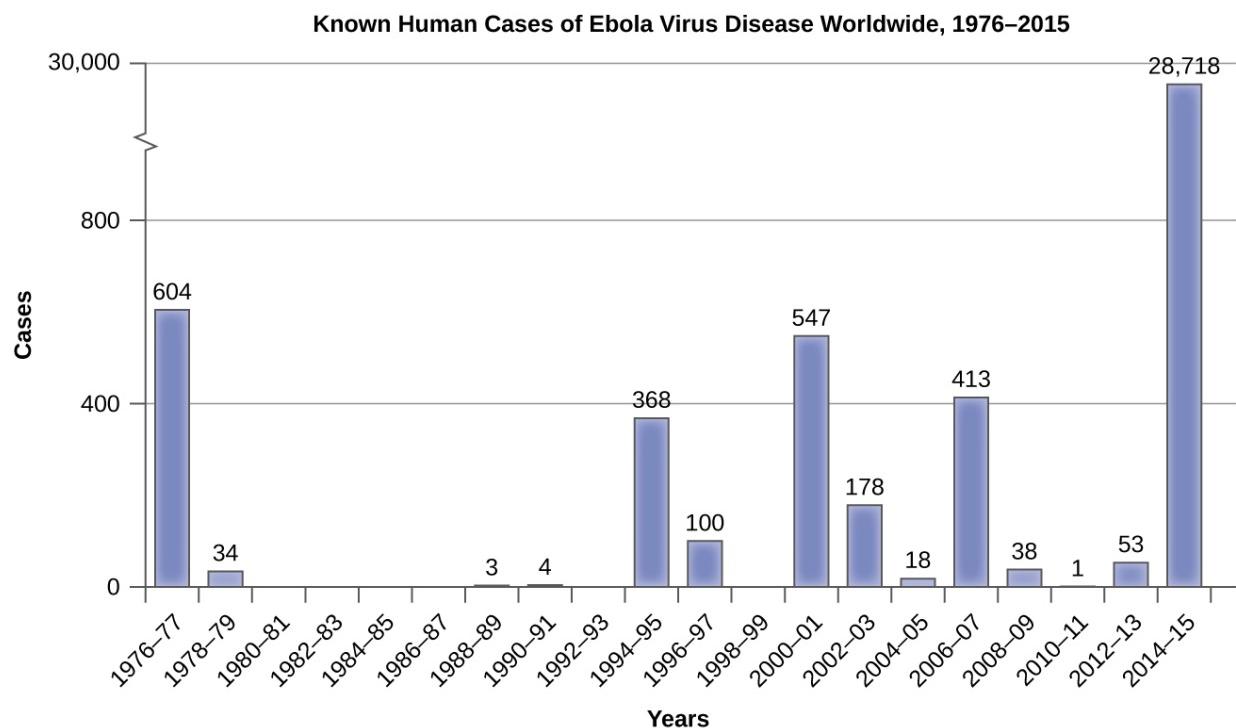
Emerging diseases may change their frequency gradually over time, or they may experience sudden epidemic growth. The importance of vigilance was made clear during the Ebola hemorrhagic fever epidemic in western Africa through 2014–2015. Although health experts had been aware of the Ebola virus since the 1970s, an outbreak on such a large scale had never happened before ([\[link\]](#)). Previous human epidemics had been small, isolated, and contained. Indeed, the gorilla and chimpanzee populations of western Africa had suffered far worse from Ebola than the human population. The pattern of small isolated human epidemics changed in 2014. Its high transmission rate, coupled with cultural practices for treatment of the dead and perhaps its emergence in an urban setting, caused the disease to spread rapidly, and thousands of people died. The international public health community responded with a large emergency effort to treat patients and contain the epidemic.

Emerging diseases are found in all countries, both developed and developing ([\[link\]](#)). Some nations are better equipped to deal with them. National and international public health agencies watch for epidemics like the Ebola outbreak in developing countries because those countries rarely have the health-care infrastructure and expertise to deal with large outbreaks effectively. Even with the support of international agencies, the systems in western Africa struggled to identify and care for the sick and control spread. In addition to the altruistic goal of saving lives and assisting nations lacking in resources, the global

nature of transportation means that an outbreak anywhere can spread quickly to every corner of the planet. Managing an epidemic in one location—its source—is far easier than fighting it on many fronts.

Ebola is not the only disease that needs to be monitored in the global environment. In 2015, WHO set priorities on several emerging diseases that had a high probability of causing epidemics and that were poorly understood (and thus urgently required research and development efforts).

A **reemerging infectious disease** is a disease that is increasing in frequency after a previous period of decline. Its reemergence may be a result of changing conditions or old prevention regimes that are no longer working. Examples of such diseases are drug-resistant forms of tuberculosis, bacterial pneumonia, and malaria. Drug-resistant strains of the bacteria causing gonorrhea and syphilis are also becoming more widespread, raising concerns of untreatable infections.



Even before the Ebola epidemic of 2014–15, Ebola was considered an emerging disease because of several smaller outbreaks between the mid-1990s and 2000s.

Some Emerging and Reemerging Infectious Diseases

Disease	Pathogen	Year Discovered	Affected Regions	Transmission
AIDS	HIV	1981	Worldwide	Contact with infected body fluids
Chikungunya fever	Chikungunya virus	1952	Africa, Asia, India; spreading to Europe and the Americas	Mosquito-borne
Ebola virus disease	Ebola virus	1976	Central and Western Africa	Contact with infected body fluids
H1N1 Influenza (swine flu)	H1N1 virus	2009	Worldwide	Droplet transmission
Lyme disease	<i>Borrelia burgdorferi</i> bacterium	1981	Northern hemisphere	From mammal reservoirs to humans by tick vectors
West Nile virus disease	West Nile virus	1937	Africa, Australia, Canada to Venezuela, Europe, Middle East, Western Asia	Mosquito-borne

Note:

- Explain why it is important to monitor emerging infectious diseases.
- Explain how a bacterial disease could reemerge, even if it had previously been successfully treated and controlled.

Note:**SARS Outbreak and Identification**

On November 16, 2002, the first case of a SARS outbreak was reported in Guangdong Province, China. The patient exhibited influenza-like symptoms such as fever, cough, myalgia, sore throat, and shortness of breath. As the number of cases grew, the Chinese government was reluctant to openly communicate information about the epidemic with the World Health Organization (WHO) and the international community. The slow reaction of Chinese public health officials to this new disease contributed to the spread of the epidemic within and later outside China. In April 2003, the Chinese government finally responded with a huge public health effort involving quarantines, medical checkpoints, and massive cleaning projects. Over 18,000 people were quarantined in Beijing alone. Large funding initiatives were created to improve health-care facilities, and dedicated outbreak teams were created to coordinate the response. By August 16, 2003, the last SARS patients were released from a hospital in Beijing nine months after the first case was reported in China.

In the meantime, SARS spread to other countries on its way to becoming a global pandemic. Though the infectious agent had yet to be identified, it was thought to be an influenza virus. The disease was named SARS, an acronym for severe acute respiratory syndrome, until the etiologic agent could be identified. Travel restrictions to Southeast Asia were enforced by many countries. By the end of the outbreak, there were 8,098 cases and 774 deaths worldwide. China and Hong Kong were hit hardest by the epidemic, but Taiwan, Singapore, and Toronto, Canada, also saw significant numbers of cases.

Fortunately, timely public health responses in many countries effectively suppressed the outbreak and led to its eventual containment. For example, the disease was introduced to Canada in February 2003 by an infected traveler from Hong Kong, who died shortly after being hospitalized. By the end of March, hospital isolation and home quarantine procedures were in place in the Toronto area, stringent anti-infection protocols were introduced in hospitals, and the media were actively reporting on the disease. Public health officials tracked down contacts of infected individuals and quarantined them. A total of 25,000 individuals were quarantined in the city. Thanks to the vigorous response of the Canadian public health community, SARS was brought under control in Toronto by June, a mere four months after it was introduced.

In 2003, WHO established a collaborative effort to identify the causative agent of SARS, which has now been identified as a coronavirus that was associated with horseshoe bats. The genome of the SARS virus was sequenced and published by researchers at the CDC and in Canada in May 2003, and in the same month researchers in the Netherlands

confirmed the etiology of the disease by fulfilling Koch's postulates for the SARS coronavirus. The last known case of SARS worldwide was reported in 2004.

Note:



This [database](#) of reports chronicles outbreaks of infectious disease around the world. It was on this system that the first information about the SARS outbreak in China emerged. The CDC publishes [*Emerging Infectious Diseases*](#), a monthly journal available online.

Key Concepts and Summary

- The **World Health Organization (WHO)** is an agency of the United Nations that collects and analyzes data on disease occurrence from member nations. WHO also coordinates public health programs and responses to international health emergencies.
- **Emerging diseases** are those that are new to human populations or that have been increasing in the past two decades. **Reemerging diseases** are those that are making a resurgence in susceptible populations after previously having been controlled in some geographic areas.

Multiple Choice

Exercise:

Problem: Which of the following would NOT be considered an emerging disease?

- a. Ebola hemorrhagic fever
- b. West Nile virus fever/encephalitis
- c. Zika virus disease
- d. Tuberculosis

Solution:

D

Exercise:

Problem: Which of the following would NOT be considered a reemerging disease?

- a. Drug-resistant tuberculosis
 - b. Drug-resistant gonorrhea
 - c. Malaria
 - d. West Nile virus fever/encephalitis
-

Solution:

D

Exercise:

Problem: Which of the following factors can lead to reemergence of a disease?

- a. A mutation that allows it to infect humans
 - b. A period of decline in vaccination rates
 - c. A change in disease reporting procedures
 - d. Better education on the signs and symptoms of the disease
-

Solution:

B

Exercise:

Problem:

Why are emerging diseases with very few cases the focus of intense scrutiny?

- a. They tend to be more deadly
 - b. They are increasing and therefore not controlled
 - c. They naturally have higher transmission rates
 - d. They occur more in developed countries
-

Solution:

B

Fill in the Blank

Exercise:

Problem:

The _____ collects data and conducts epidemiologic studies at the global level.

Solution:

WHO (World Health Organization)

Critical Thinking

Exercise:

Problem:

An Atlantic crossing by boat from England to New England took 60–80 days in the 18th century. In the late 19th century the voyage took less than a week. How do you think these time differences for travel might have impacted the spread of infectious diseases from Europe to the Americas, or vice versa?

Innate Immunity - Introduction

class="introduction"

Varicella, or chickenpox, is caused by the highly contagious varicella-zoster virus.

The characteristic rash seen here is partly a result of inflammation associated with the body's immune response to the virus.

Inflammation is a response mechanism of innate immunity that helps the body fight off a wide range of infections.

(credit:
modification
of work by
Centers for

Disease
Control and
Prevention)



Despite relatively constant exposure to pathogenic microbes in the environment, humans do not generally suffer from constant infection or disease. Under most circumstances, the body is able to defend itself from the threat of infection thanks to a complex immune system designed to repel, kill, and expel disease-causing invaders. Immunity as a whole can be described as two interrelated parts: nonspecific innate immunity, which is the subject of this chapter, and specific adaptive host defenses, which are discussed in the next chapter.

The nonspecific innate immune response provides a first line of defense that can often prevent infections from gaining a solid foothold in the body. These defenses are described as *nonspecific* because they do not target any specific pathogen; rather, they defend against a wide range of potential pathogens. They are called *innate* because they are built-in mechanisms of the human organism. Unlike the specific adaptive defenses, they are not acquired over time and they have no “memory” (they do not improve after repeated exposures to specific pathogens).

Broadly speaking, nonspecific innate defenses provide an immediate (or very rapid) response against potential pathogens. However, these responses are neither perfect nor impenetrable. They can be circumvented by pathogens on occasion, and sometimes they can even cause damage to the body, contributing to the signs and symptoms of infection ([\[link\]](#)).

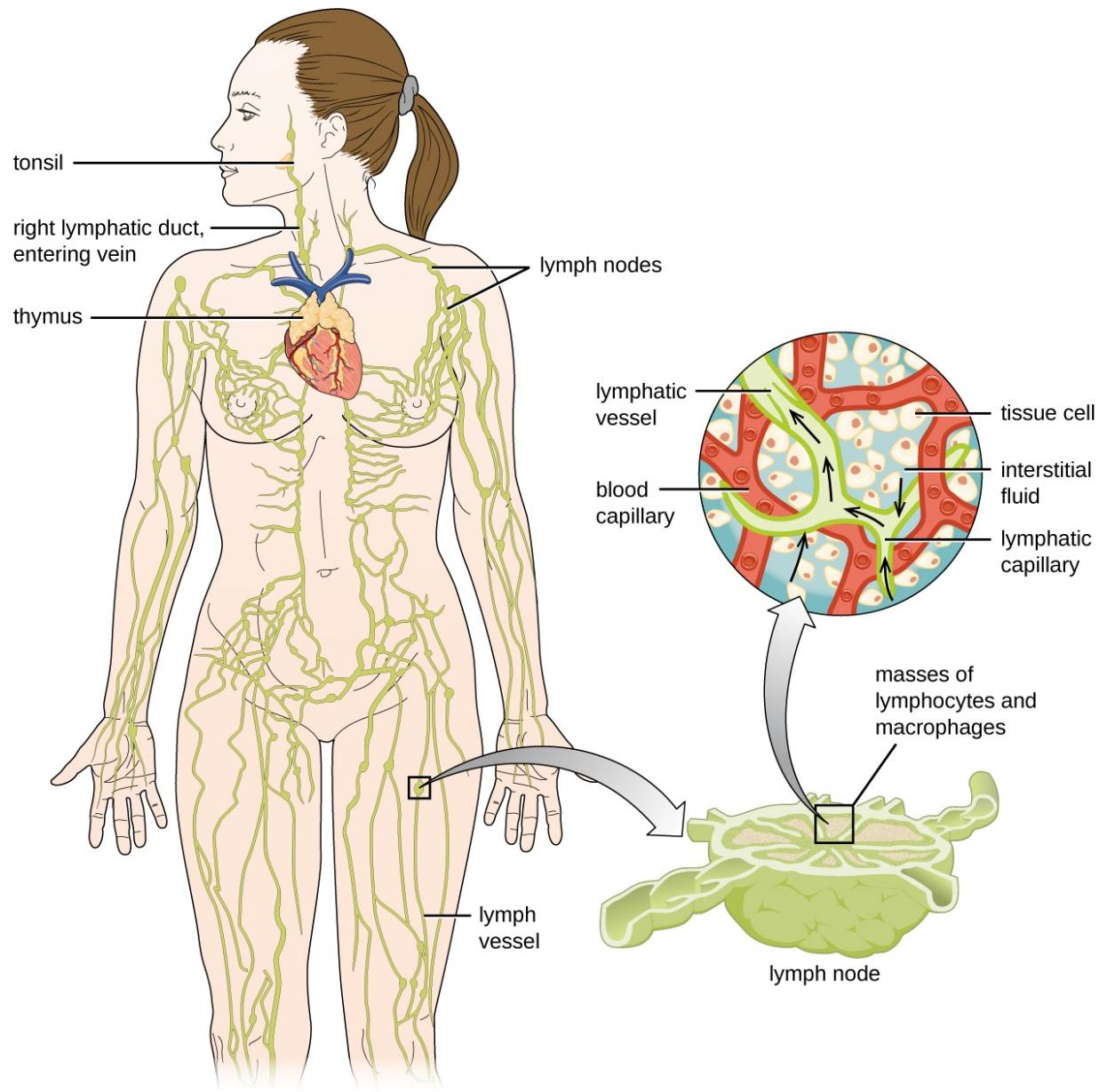
Lymphatic System

LEARNING OBJECTIVES

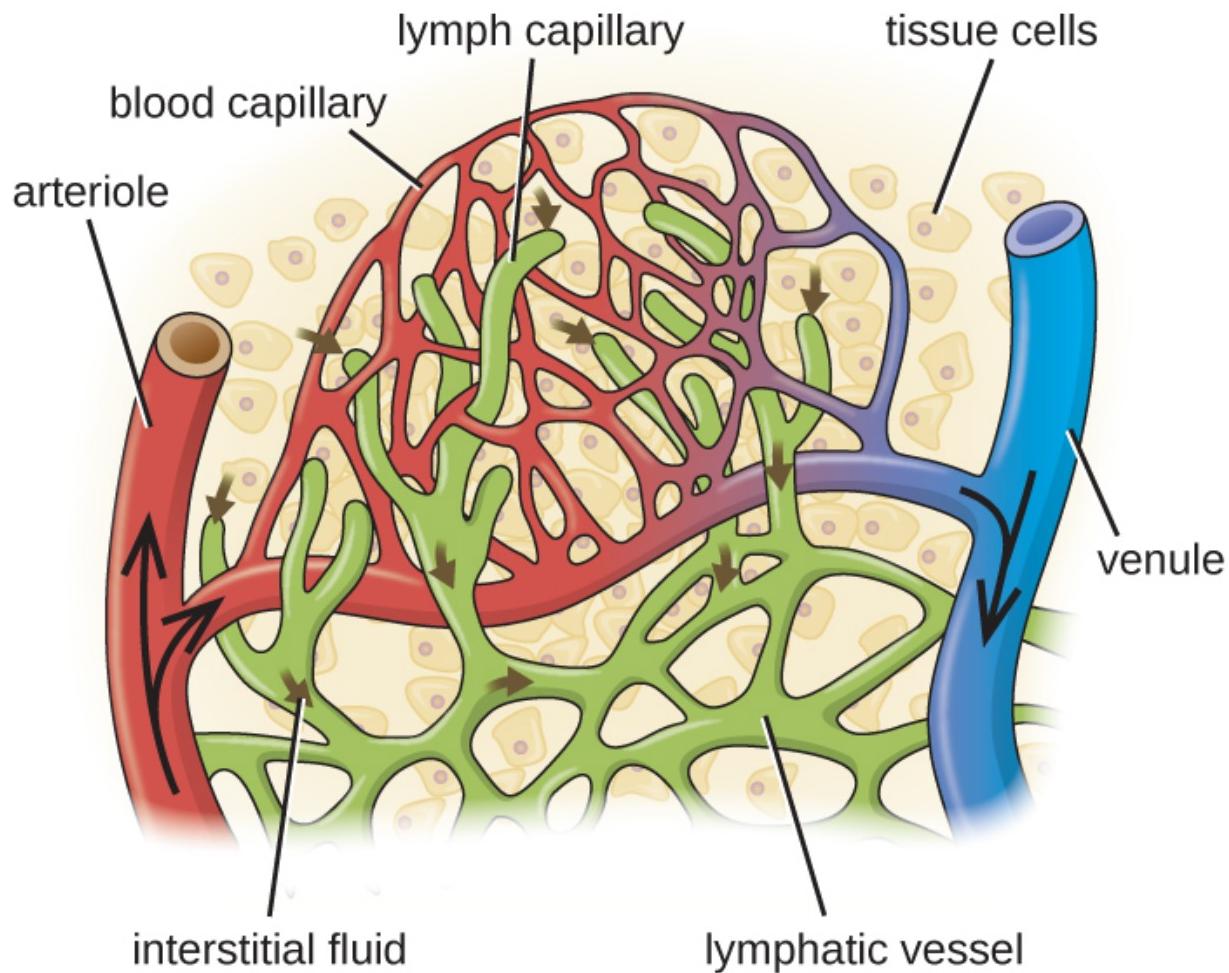
- Describe the major anatomical features of the lymphatic system
- Explain why the lymphatic system lack normal microbiota
- Explain how microorganisms overcome defenses of the lymphatic systems to cause infection

The Lymphatic System

The lymphatic system is also a network of vessels that run throughout the body ([\[link\]](#)). However, these vessels do not form a full circulating system and are not pressurized by the heart. Rather, the lymphatic system is an open system with the fluid moving in one direction from the extremities toward two drainage points into veins just above the heart. Lymphatic fluids move more slowly than blood because they are not pressurized. Small lymph capillaries interact with blood capillaries in the interstitial spaces in tissues. Fluids from the tissues enter the lymph capillaries and are drained away ([\[link\]](#)). These fluids, termed lymph, also contain large numbers of white blood cells. The three major functions of the lymphatic system are: (1) to collect excess fluid from the inter-cellular spaces, (2) to transport digested fats to the circulatory system, and (3) to provide innate and adaptive defense against infections.



The essential components of the human lymphatic system drain fluid away from tissues.

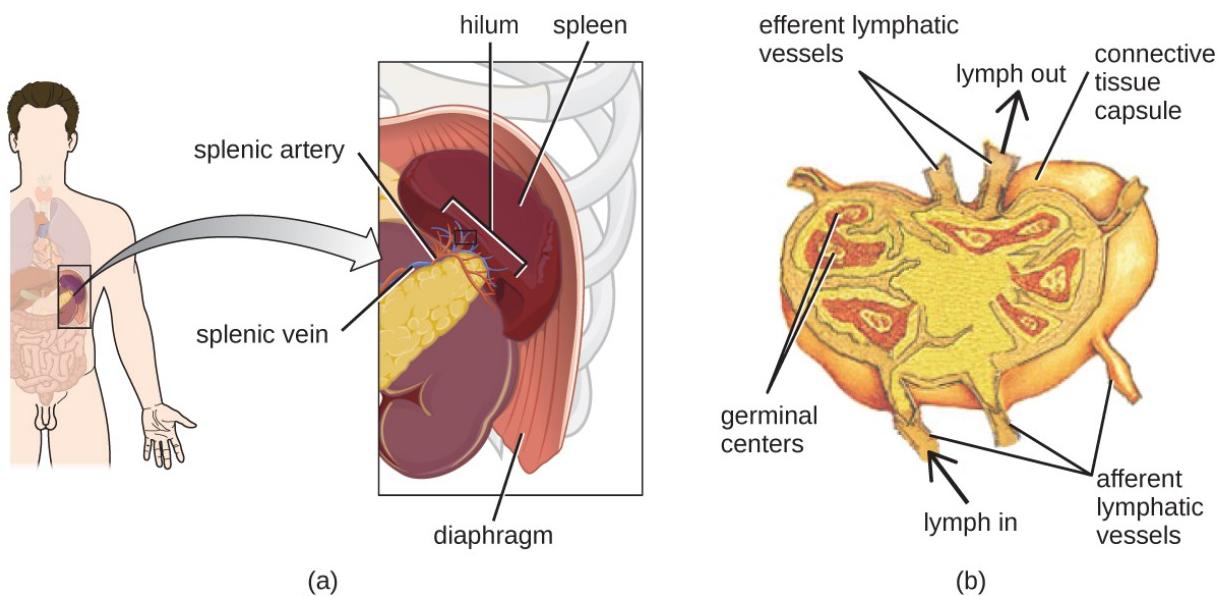


Blood enters the capillaries from an arteriole (red) and leaves through venules (blue). Interstitial fluids may drain into the lymph capillaries (green) and proceed to lymph nodes. (credit: modification of work by National Cancer Institute, National Institutes of Health)

The lymphatic system contains two types of lymphoid tissues. The **primary lymphoid tissue** includes bone marrow and the thymus. Bone marrow contains the hematopoietic stem cells (HSC) that differentiate and mature into the various types of blood cells and lymphocytes (see [\[link\]](#)). The **thymus**, a multilobed gland, is located below the sternum and is directly connected with the adaptive defense system considering it is the site of maturation for T cells. We will talk more about this on the adaptive

defenses chapter, but it is interesting to notice that the thymus reduces its size as we age, and is replaced by connective tissue and fats. The **secondary lymphoid tissues** include the spleen, lymph nodes, and several areas of diffuse lymphoid tissues underlying epithelial membranes. The **spleen**, an encapsulated structure, filters blood and captures pathogens and antigens that pass into it ([\[link\]](#)). The spleen contains specialized macrophages and dendritic cells that are crucial for antigen presentation, a mechanism critical for activation of T lymphocytes and B lymphocytes (see [Major Histocompatibility Complexes and Antigen-Presenting Cells](#)).

Lymph nodes are bean-shaped organs situated throughout the body. These structures contain areas called germinal centers that are rich in B and T lymphocytes. The **lymph nodes** also contain macrophages and dendritic cells for antigen presentation. Lymph from nearby tissues enters the lymph node through afferent lymphatic vessels and encounters these lymphocytes as it passes through; the lymph exits the lymph node through the efferent lymphatic vessels ([\[link\]](#)).



- (a) The spleen is a lymphatic organ located in the upper left quadrant of the abdomen near the stomach and left kidney. It contains numerous phagocytotes and lymphocytes that combat and prevent circulatory infections by killing and removing pathogens from the blood. (b) Lymph nodes are masses of lymphatic tissue located along the larger

lymph vessels. They contain numerous lymphocytes that kill and remove pathogens from lymphatic fluid that drains from surrounding tissues.

Lymphoid tissues also protect mucous membranes all over the body, such as gastrointestinal tract, lungs, salivary glands, eyes, etc, and are referred to as **mucosa-associated lymphoid tissue or MALT**. The Peyer's patches in the lining of the small intestines are example of gut-associated lymphoid tissue (GALT). Similar agglomerations found in the respiratory system are called bronchial-associated lymphoid tissue (BALT). It is important to understand that lymphoid tissues contribute to the innate defenses because it contains cells that can phagocytize microorganisms and foreign material.

Note:



The lymphatic system filters fluids that have accumulated in tissues before they are returned to the blood. A brief overview of this process is provided at [this website](#).

Note:

- What are the main functions of the lymphatic system?

Infections of the Lymphatic System

Like the circulatory system, the lymphatic system does not have a normal microbiota, and the large numbers of immune cells typically eliminate transient microbes before they can establish an infection. Only microbes with an array of virulence factors are able to overcome these defenses and establish infection in the lymphatic system. However, when a localized infection begins to spread, the lymphatic system is often the first place the invading microbes can be detected.

Infections in the lymphatic system also trigger an inflammatory response. Inflammation of lymphatic vessels, called **lymphangitis**, can produce visible red streaks under the skin. Inflammation in the lymph nodes can cause them to swell. A swollen lymph node is referred to as a **bubo**, and the condition is referred to as **lymphadenitis**.

Key Concepts and Summary

- The **lymphatic system** moves fluids from the interstitial spaces of tissues toward the circulatory system and filters the lymph.
- The lymphatic systems is home to many components of the host immune defenses.

Critical Thinking

Exercise:

Problem:

Why are there more afferent lymphatic vessels carrying lymph to the lymph nodes than there are efferent lymphatic vessels?

Physical Defenses

LEARNING OBJECTIVES

- Describe the various physical barriers and mechanical defenses that protect the human body against infection and disease
- Describe the role of microbiota as a first-line defense against infection and disease

Nonspecific innate immunity can be characterized as a multifaceted system of defenses that targets invading pathogens in a nonspecific manner. In this chapter, we have divided the numerous defenses that make up this system into three categories: physical defenses, chemical defenses, and cellular defenses. However, it is important to keep in mind that these defenses do not function independently, and the categories often overlap. [\[link\]](#) provides an overview of the nonspecific defenses discussed in this chapter.

Overview of Nonspecific Innate Immune Defenses

Physical defenses

Physical barriers

Mechanical defenses

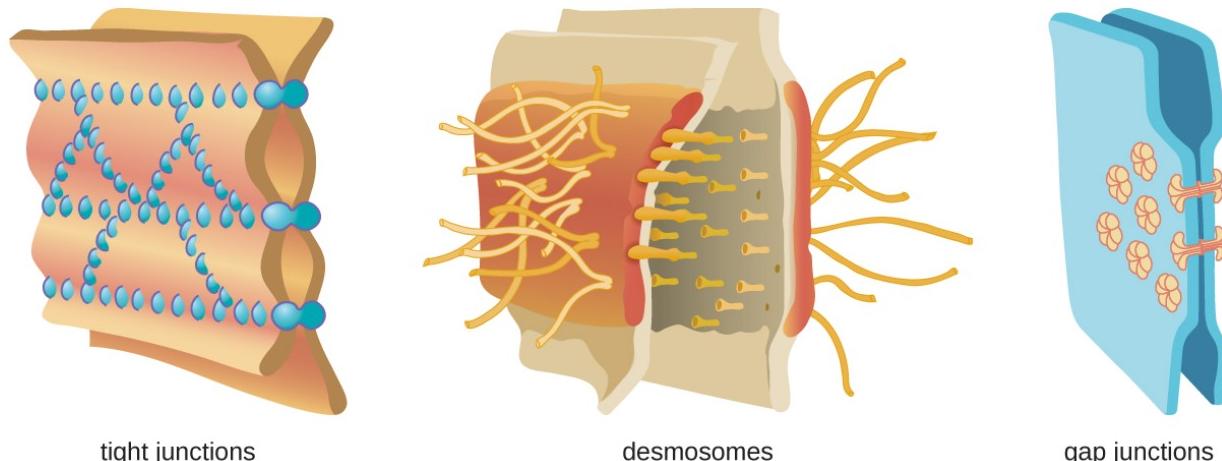
Overview of Nonspecific Innate Immune Defenses	
	Microbiome
	Chemicals and enzymes in body fluids
	Antimicrobial peptides
Chemical defenses	Plasma protein mediators
	Cytokines
	Inflammation-eliciting mediators
Cellular defenses	Granulocytes
	Agranulocytes

Physical defenses provide the body's most basic form of nonspecific defense. They include physical barriers to microbes, such as the skin and mucous membranes, as well as mechanical defenses that physically remove microbes and debris from areas of the body where they might cause harm or infection. In addition, the microbiome provides a measure of physical protection against disease, as microbes of the normal microbiota compete with pathogens for nutrients and cellular binding sites necessary to cause infection.

Physical Barriers

Physical barriers play an important role in preventing microbes from reaching tissues that are susceptible to infection. At the cellular level, barriers consist of cells that are tightly joined to prevent invaders from crossing through to deeper tissue. For example, the endothelial cells that line blood vessels have very tight cell-to-cell junctions, blocking microbes from gaining access to the bloodstream. Cell junctions are generally composed of cell membrane proteins that may connect with the

extracellular matrix or with complementary proteins from neighboring cells. Tissues in various parts of the body have different types of cell junctions. These include tight junctions, desmosomes, and gap junctions, as illustrated in [\[link\]](#). Invading microorganisms may attempt to break down these substances chemically, using enzymes such as proteases that can cause structural damage to create a point of entry for pathogens.

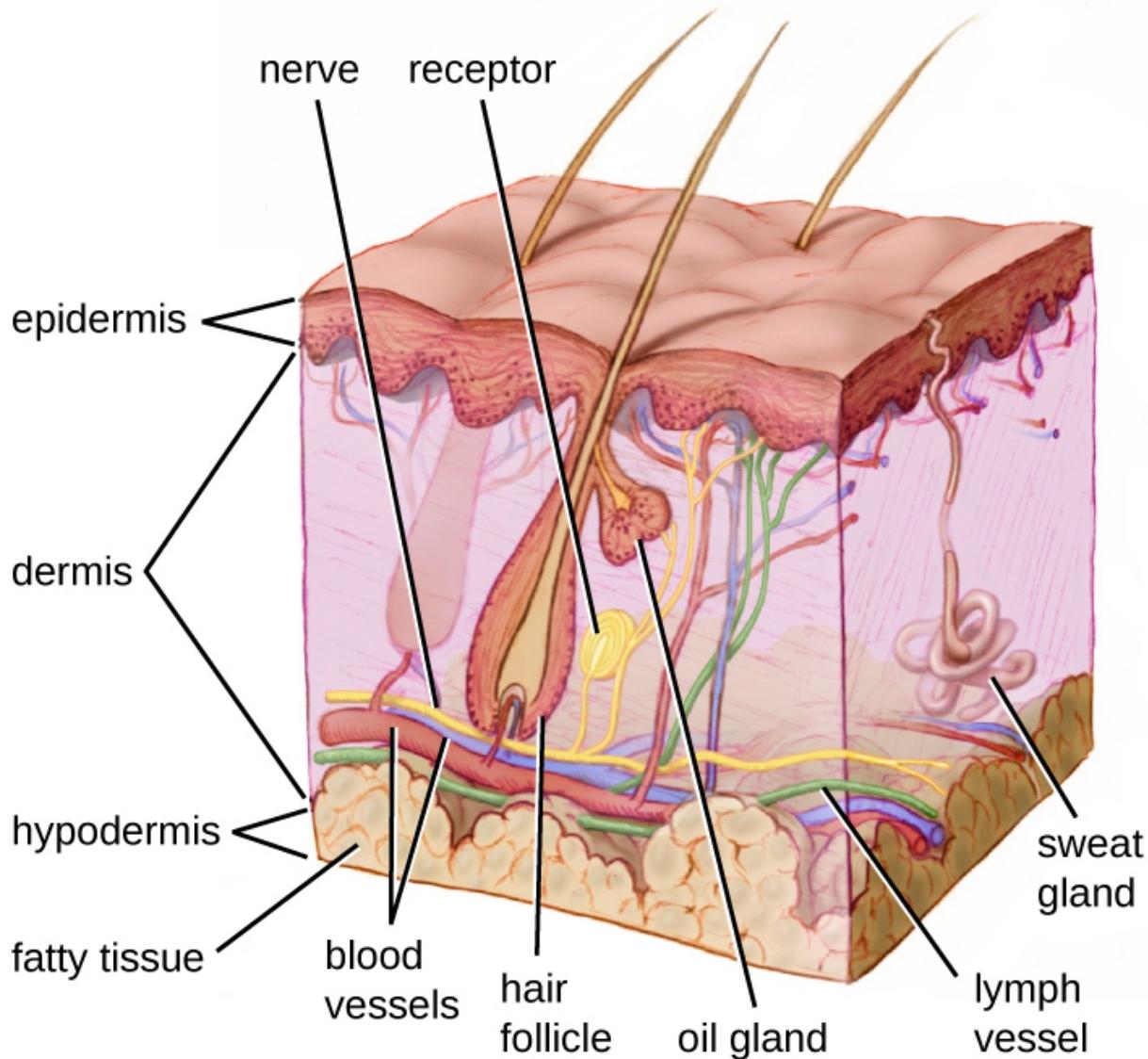


There are multiple types of cell junctions in human tissue, three of which are shown here. Tight junctions rivet two adjacent cells together, preventing or limiting material exchange through the spaces between them. Desmosomes have intermediate fibers that act like shoelaces, tying two cells together, allowing small materials to pass through the resulting spaces. Gap junctions are channels between two cells that permit their communication via signals. (credit: modification of work by Mariana Ruiz Villareal)

The Skin Barrier

One of the body's most important physical barriers is the skin barrier, which is composed of three layers of closely packed cells. The thin upper layer is called the epidermis. A second, thicker layer, called the dermis, contains

hair follicles, sweat glands, nerves, and blood vessels. A layer of fatty tissue called the hypodermis lies beneath the dermis and contains blood and lymph vessels ([\[link\]](#)).



Human skin has three layers, the epidermis, the dermis, and the hypodermis, which provide a thick barrier between microbes outside the body and deeper tissues. Dead skin cells on the surface of the epidermis are continually shed, taking with them microbes on the skin's surface. (credit: modification of work by National Institutes of Health)

The topmost layer of skin, the epidermis, consists of cells that are packed with keratin. These dead cells remain as a tightly connected, dense layer of protein-filled cell husks on the surface of the skin. The keratin makes the skin's surface mechanically tough and resistant to degradation by bacterial enzymes. Fatty acids on the skin's surface create a dry, salty, and acidic environment that inhibits the growth of some microbes and is highly resistant to breakdown by bacterial enzymes. In addition, the dead cells of the epidermis are frequently shed, along with any microbes that may be clinging to them. Shed skin cells are continually replaced with new cells from below, providing a new barrier that will soon be shed in the same way.

Infections can occur when the skin barrier is compromised or broken. A wound can serve as a point of entry for opportunistic pathogens, which can infect the skin tissue surrounding the wound and possibly spread to deeper tissues.

Note:

Every Rose Has its Thorn

Mike, a gardener from southern California, recently noticed a small red bump on his left forearm. Initially, he did not think much of it, but soon it grew larger and then ulcerated (opened up), becoming a painful lesion that extended across a large part of his forearm ([\[link\]](#)). He went to an urgent care facility, where a physician asked about his occupation. When he said he was a landscaper, the physician immediately suspected a case of sporotrichosis, a type of fungal infection known as rose gardener's disease because it often afflicts landscapers and gardening enthusiasts.

Under most conditions, fungi cannot produce skin infections in healthy individuals. Fungi grow filaments known as hyphae, which are not particularly invasive and can be easily kept at bay by the physical barriers of the skin and mucous membranes. However, small wounds in the skin, such as those caused by thorns, can provide an opening for opportunistic pathogens like *Sporothrix schenkii*, a soil-dwelling fungus and the causative agent of rose gardener's disease. Once it breaches the skin

barrier, *S. schenckii* can infect the skin and underlying tissues, producing ulcerated lesions like Mike's. Compounding matters, other pathogens may enter the infected tissue, causing secondary bacterial infections.

Luckily, rose gardener's disease is treatable. Mike's physician wrote him a prescription for some antifungal drugs as well as a course of antibiotics to combat secondary bacterial infections. His lesions eventually healed, and Mike returned to work with a new appreciation for gloves and protective clothing.

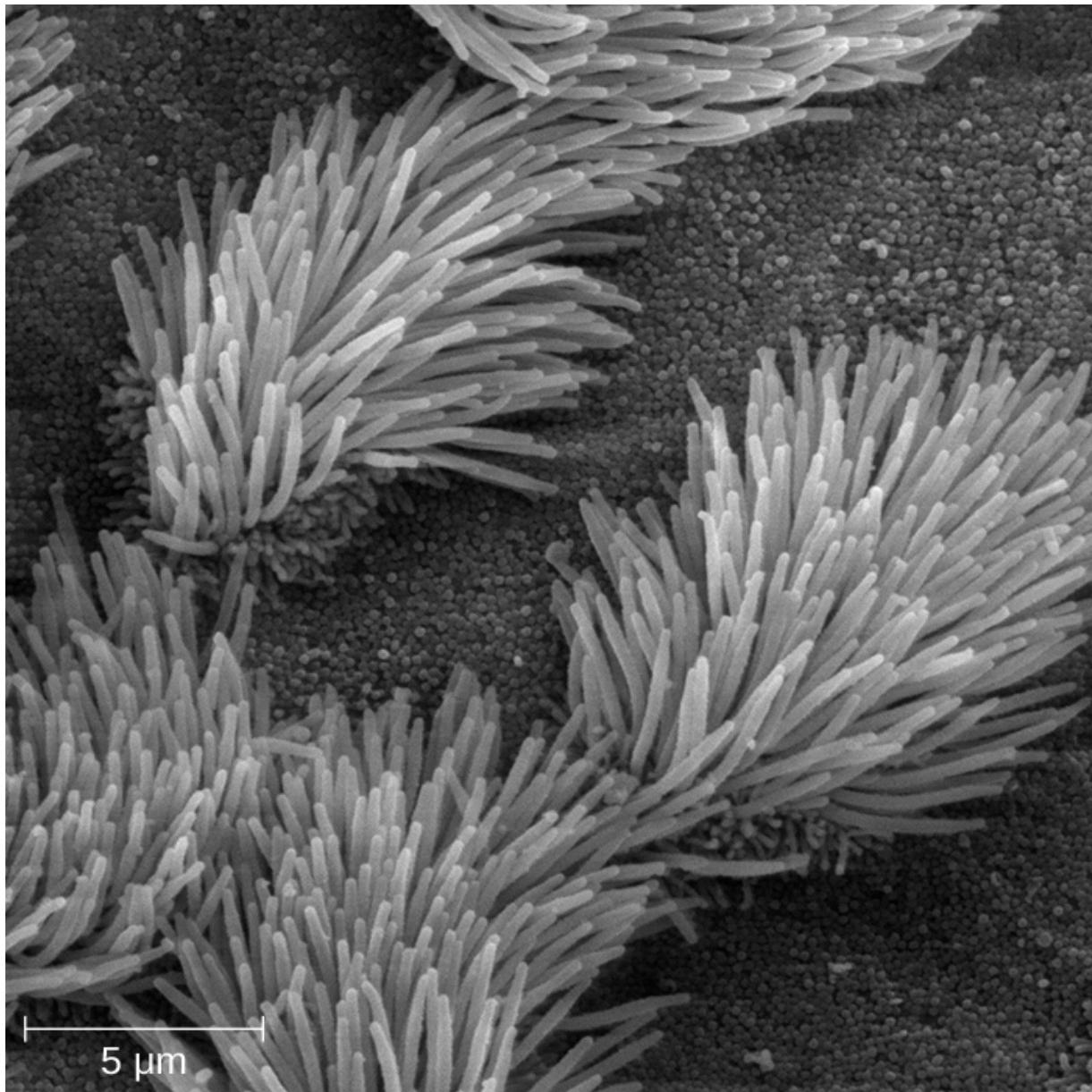


Rose gardener's disease can occur when the fungus *Sporothrix schenckii* breaches the skin through small cuts, such as might be inflicted by thorns. (credit left: modification of work by Elisa Self; credit right: modification of work by Centers for Disease Control and Prevention)

Mucous Membranes

The **mucous membranes** lining the nose, mouth, lungs, and urinary and digestive tracts provide another nonspecific barrier against potential pathogens. Mucous membranes consist of a layer of epithelial cells bound by tight junctions. The epithelial cells secrete a moist, sticky substance called **mucus**, which covers and protects the more fragile cell layers beneath it and traps debris and particulate matter, including microbes. Mucus secretions also contain antimicrobial peptides.

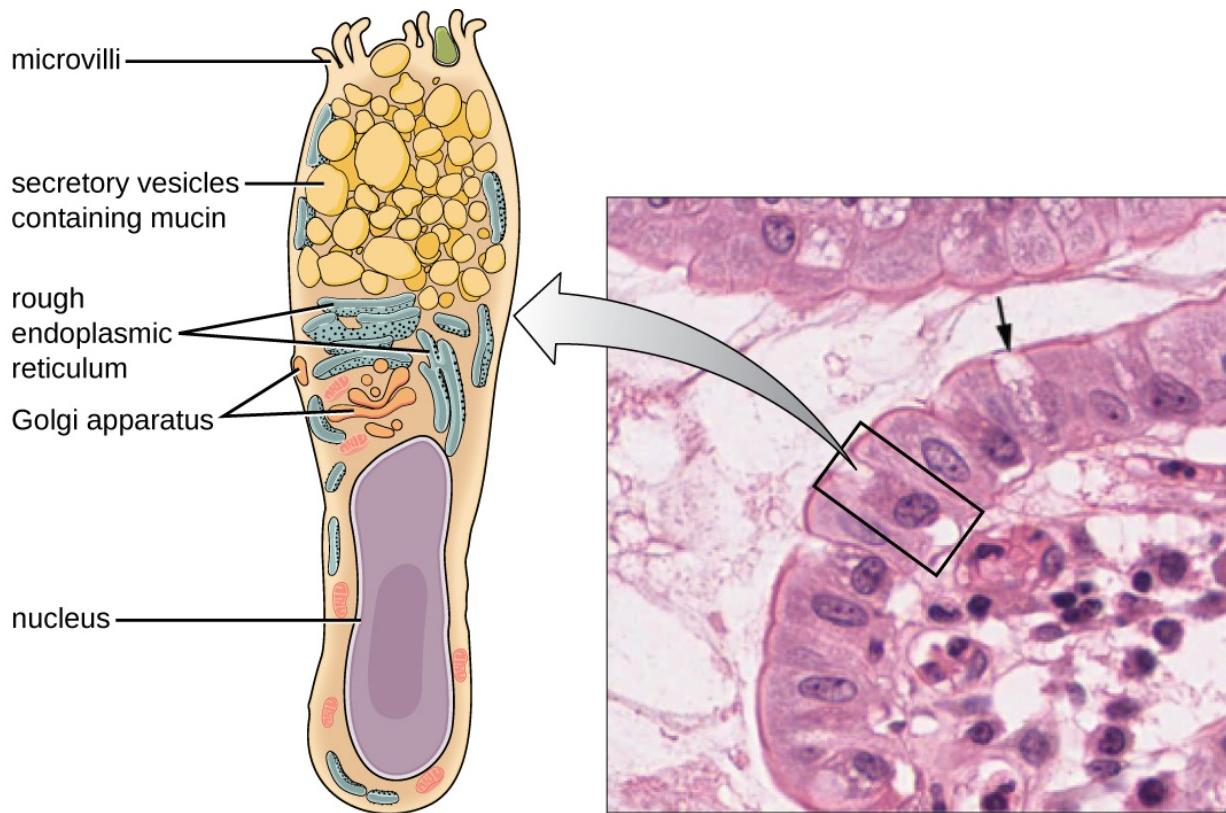
In many regions of the body, mechanical actions serve to flush mucus (along with trapped or dead microbes) out of the body or away from potential sites of infection. For example, in the respiratory system, inhalation can bring microbes, dust, mold spores, and other small airborne debris into the body. This debris becomes trapped in the mucus lining the respiratory tract, a layer known as the mucociliary blanket. The epithelial cells lining the upper parts of the respiratory tract are called **ciliated epithelial cells** because they have hair-like appendages known as cilia. Movement of the cilia propels debris-laden mucus out and away from the lungs. The expelled mucus is then swallowed and destroyed in the stomach, or coughed up, or sneezed out ([\[link\]](#)). This system of removal is often called the **mucociliary escalator**.



This scanning electron micrograph shows ciliated and nonciliated epithelial cells from the human trachea. The mucociliary escalator pushes mucus away from the lungs, along with any debris or microorganisms that may be trapped in the sticky mucus, and the mucus moves up to the esophagus where it can be removed by swallowing.

The mucociliary escalator is such an effective barrier to microbes that the lungs, the lowermost (and most sensitive) portion of the respiratory tract, were long considered to be a sterile environment in healthy individuals. Only recently has research suggested that healthy lungs may have a small normal microbiota. Disruption of the mucociliary escalator by the damaging effects of smoking or diseases such as cystic fibrosis can lead to increased colonization of bacteria in the lower respiratory tract and frequent infections, which highlights the importance of this physical barrier to host defenses.

Like the respiratory tract, the digestive tract is a portal of entry through which microbes enter the body, and the mucous membranes lining the digestive tract provide a nonspecific physical barrier against ingested microbes. The intestinal tract is lined with epithelial cells, interspersed with mucus-secreting goblet cells ([\[link\]](#)). This mucus mixes with material received from the stomach, trapping foodborne microbes and debris. The mechanical action of **peristalsis**, a series of muscular contractions in the digestive tract, moves the sloughed mucus and other material through the intestines, rectum, and anus, excreting the material in feces.



Goblet cells produce and secrete mucus. The arrows in this micrograph point to the mucus-secreting goblet cells (magnification $1600\times$) in the intestinal epithelium. (credit micrograph: Micrograph provided by the Regents of University of Michigan Medical School © 2012)

Endothelia

The epithelial cells lining the urogenital tract, blood vessels, lymphatic vessels, and certain other tissues are known as **endothelia**. These tightly packed cells provide a particularly effective frontline barrier against invaders. The endothelia of the **blood-brain barrier**, for example, protect the central nervous system (CNS), which consists of the brain and the spinal cord. The CNS is one of the most sensitive and important areas of the body, as microbial infection of the CNS can quickly lead to serious and often fatal inflammation. The cell junctions in the blood vessels traveling through the

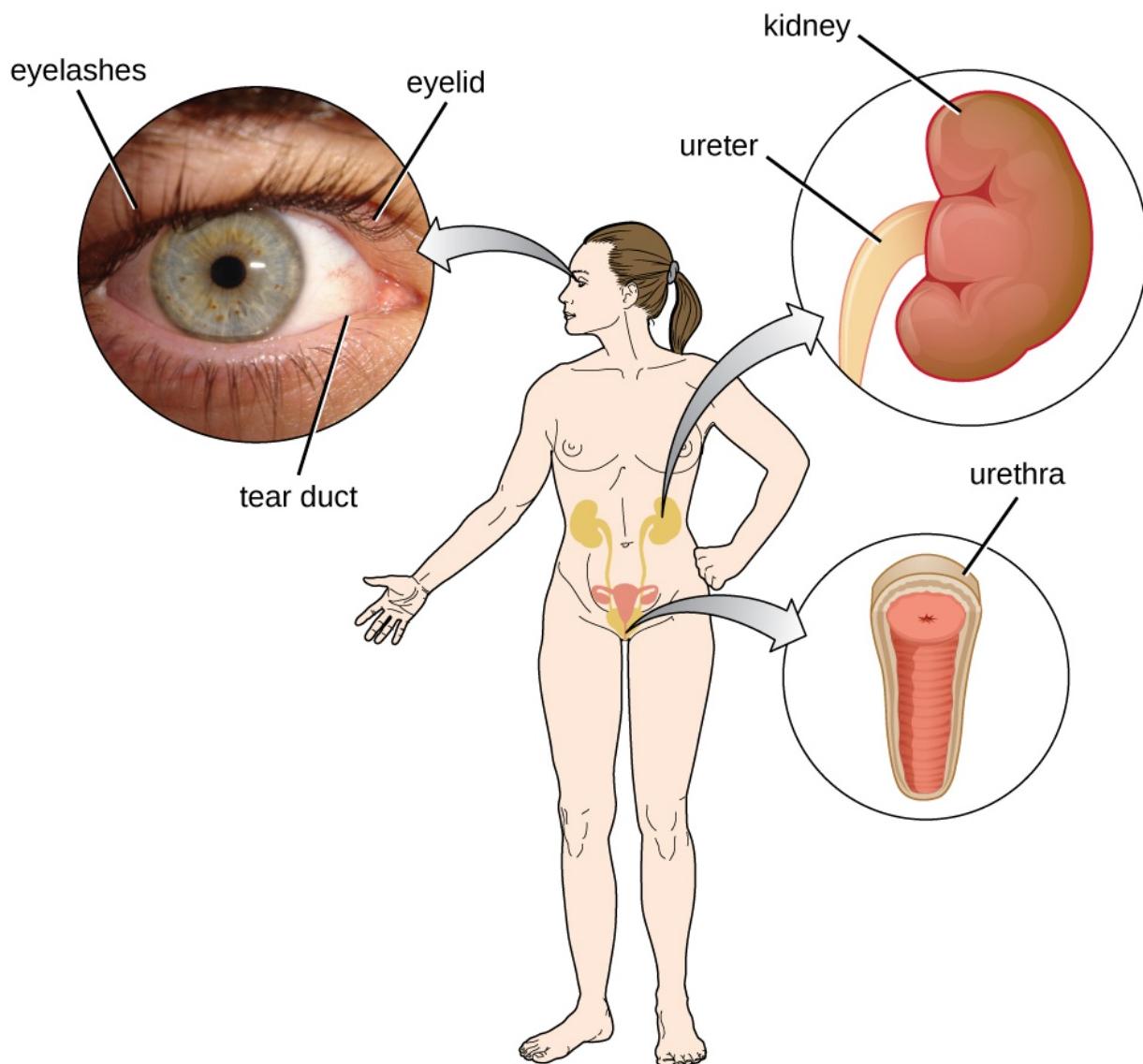
CNS are some of the tightest and toughest in the body, preventing any transient microbes in the bloodstream from entering the CNS. This keeps the cerebrospinal fluid that surrounds and bathes the brain and spinal cord sterile under normal conditions.

Note:

- Describe how the mucociliary escalator functions.
- Name two places you would find endothelia.

Mechanical Defenses

In addition to physical barriers that keep microbes out, the body has a number of mechanical defenses that physically remove pathogens from the body, preventing them from taking up residence. We have already discussed several examples of mechanical defenses, including the shedding of skin cells, the expulsion of mucus via the mucociliary escalator, and the excretion of feces through intestinal peristalsis. Other important examples of mechanical defenses include the flushing action of urine and tears, which both serve to carry microbes away from the body. The flushing action of urine is largely responsible for the normally sterile environment of the urinary tract, which includes the kidneys, ureters, and urinary bladder. Urine passing out of the body washes out transient microorganisms, preventing them from taking up residence. The eyes also have physical barriers and mechanical mechanisms for preventing infections. The eyelashes and eyelids prevent dust and airborne microorganisms from reaching the surface of the eye. Any microbes or debris that make it past these physical barriers may be flushed out by the mechanical action of blinking, which bathes the eye in tears, washing debris away ([\[link\]](#)).



Tears flush microbes away from the surface of the eye. Urine washes microbes out of the urinary tract as it passes through; as a result, the urinary system is normally sterile.

Note:

- Name two mechanical defenses that protect the eyes.

Microbiome

In various regions of the body, resident microbiota serve as an important first-line defense against invading pathogens. Through their occupation of cellular binding sites and competition for available nutrients, the resident microbiota prevent the critical early steps of pathogen attachment and proliferation required for the establishment of an infection. For example, in the vagina, members of the resident microbiota compete with opportunistic pathogens like the yeast *Candida*. This competition prevents infections by limiting the availability of nutrients, thus inhibiting the growth of *Candida*, keeping its population in check. Similar competitions occur between the microbiota and potential pathogens on the skin, in the upper respiratory tract, and in the gastrointestinal tract. As will be discussed later in this chapter, the resident microbiota also contribute to the chemical defenses of the innate nonspecific host defenses.

The importance of the normal microbiota in host defenses is highlighted by the increased susceptibility to infectious diseases when the microbiota is disrupted or eliminated. Treatment with antibiotics can significantly deplete the normal microbiota of the gastrointestinal tract, providing an advantage for pathogenic bacteria to colonize and cause diarrheal infection. In the case of diarrhea caused by *Clostridium difficile*, the infection can be severe and potentially lethal. One strategy for treating *C. difficile* infections is fecal transplantation, which involves the transfer of fecal material from a donor (screened for potential pathogens) into the intestines of the recipient patient as a method of restoring the normal microbiota and combating *C. difficile* infections.

[[link](#)] provides a summary of the physical defenses discussed in this section.

Physical Defenses of Nonspecific Innate Immunity

Physical Defense	Example	Nonspecific Innate Immunity Function
Defense	Examples	Function
Cellular barriers	Skin, mucous membranes, endothelial cells	Deny entry to pathogens
Mechanical defenses	Shedding of skin cells, mucociliary sweeping, peristalsis, flushing action of urine and tears	Remove pathogens from potential sites of infection
Microbiome	Resident bacteria of the skin, upper respiratory tract, gastrointestinal tract, and genitourinary tract	Compete with pathogens for cellular binding sites and nutrients

Note:

- List two ways resident microbiota defend against pathogens.

Key Concepts and Summary

- **Nonspecific innate immunity** provides a first line of defense against infection by nonspecifically blocking entry of microbes and targeting them for destruction or removal from the body.
- The physical defenses of innate immunity include physical barriers, mechanical actions that remove microbes and debris, and the microbiome, which competes with and inhibits the growth of pathogens.

- The skin, mucous membranes, and endothelia throughout the body serve as physical barriers that prevent microbes from reaching potential sites of infection. Tight cell junctions in these tissues prevent microbes from passing through.
- Microbes trapped in dead skin cells or **mucus** are removed from the body by mechanical actions such as shedding of skin cells, mucociliary sweeping, coughing, **peristalsis**, and flushing of bodily fluids (e.g., urination, tears)
- The resident microbiota provide a physical defense by occupying available cellular binding sites and competing with pathogens for available nutrients.

Short Answer

Exercise:

Problem:

Differentiate a physical barrier from a mechanical removal mechanism and give an example of each.

Exercise:

Problem:

Identify some ways that pathogens can breach the physical barriers of the innate immune system.

Chemical Defenses

LEARNING OBJECTIVES

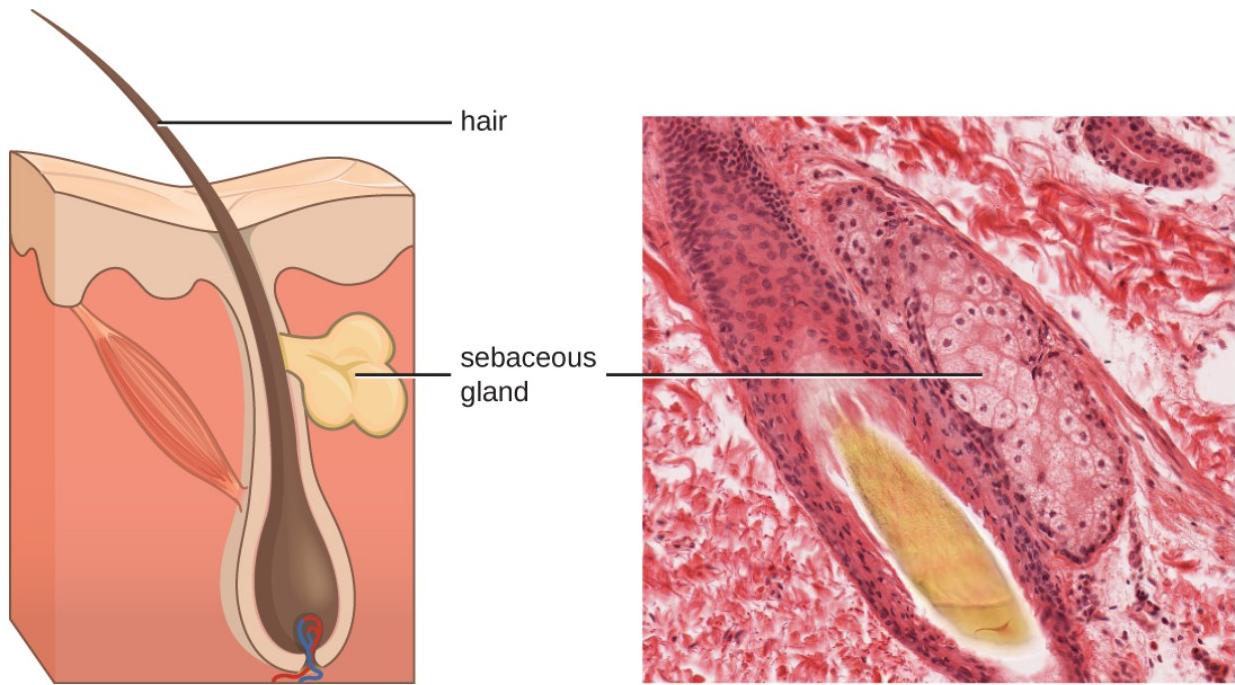
- Describe how enzymes in body fluids provide protection against infection or disease
- List and describe the function of antimicrobial peptides, complement components, cytokines, and acute-phase proteins
- Describe similarities and differences among classic, alternate, and lectin complement pathways

In addition to physical defenses, the innate nonspecific immune system uses a number of **chemical mediators** that inhibit microbial invaders. The term “chemical mediators” encompasses a wide array of substances found in various body fluids and tissues throughout the body. Chemical mediators may work alone or in conjunction with each other to inhibit microbial colonization and infection.

Some chemical mediators are endogenously produced, meaning they are produced by human body cells; others are produced exogenously, meaning that they are produced by certain microbes that are part of the microbiome. Some mediators are produced continually, bathing the area in the antimicrobial substance; others are produced or activated primarily in response to some stimulus, such as the presence of microbes.

Chemical and Enzymatic Mediators Found in Body Fluids

Fluids produced by the skin include examples of both endogenous and exogenous mediators. Sebaceous glands in the dermis secrete an oil called sebum that is released onto the skin surface through hair follicles. This sebum is an endogenous mediator, providing an additional layer of defense by helping seal off the pore of the hair follicle, preventing bacteria on the skin’s surface from invading sweat glands and surrounding tissue ([\[link\]](#)). Certain members of the microbiome, such as the bacterium *Propionibacterium acnes* and the fungus *Malassezia*, among others, can use lipase enzymes to degrade sebum, using it as a food source. This produces oleic acid, which creates a mildly acidic environment on the surface of the skin that is inhospitable to many pathogenic microbes. Oleic acid is an example of an exogenously produced mediator because it is produced by resident microbes and not directly by body cells.



Sebaceous glands secrete sebum, a chemical mediator that lubricates and protects the skin from invading microbes. Sebum is also a food source for resident microbes that produce oleic acid, an exogenously produced mediator. (credit micrograph: Micrograph provided by the Regents of University of Michigan Medical School © 2012)

Environmental factors that affect the microbiota of the skin can have a direct impact on the production of chemical mediators. Low humidity or decreased sebum production, for example, could make the skin less habitable for microbes that produce oleic acid, thus making the skin more susceptible to pathogens normally inhibited by the skin's low pH. Many skin moisturizers are formulated to counter such effects by restoring moisture and essential oils to the skin.

The digestive tract also produces a large number of chemical mediators that inhibit or kill microbes. In the oral cavity, saliva contains mediators such as lactoperoxidase enzymes, and mucus secreted by the esophagus contains the antibacterial enzyme lysozyme. In the stomach, highly acidic gastric fluid kills most microbes. In the lower digestive tract, the intestines have pancreatic and intestinal enzymes, antibacterial peptides (cryptins), bile produced from the liver, and specialized Paneth cells that produce lysozyme. Together, these mediators are able to eliminate most pathogens that manage to survive the acidic environment of the stomach.

In the urinary tract, urine flushes microbes out of the body during urination. Furthermore, the slight acidity of urine (the average pH is about 6) inhibits the growth of many microbes

and potential pathogens in the urinary tract.

The female reproductive system employs lactate, an exogenously produced chemical mediator, to inhibit microbial growth. The cells and tissue layers composing the vagina produce glycogen, a branched and more complex polymer of glucose. Lactobacilli in the area ferment glycogen to produce lactate, lowering the pH in the vagina and inhibiting transient microbiota, opportunistic pathogens like *Candida* (a yeast associated with vaginal infections), and other pathogens responsible for sexually transmitted diseases.

In the eyes, tears contain the chemical mediators lysozyme and lactoferrin, both of which are capable of eliminating microbes that have found their way to the surface of the eyes. Lysozyme cleaves the bond between NAG and NAM in peptidoglycan, a component of the cell wall in bacteria. It is more effective against gram-positive bacteria, which lack the protective outer membrane associated with gram-negative bacteria. Lactoferrin inhibits microbial growth by chemically binding and sequestering iron. This effectually starves many microbes that require iron for growth.

In the ears, cerumen (earwax) exhibits antimicrobial properties due to the presence of fatty acids, which lower the pH to between 3 and 5.

The respiratory tract uses various chemical mediators in the nasal passages, trachea, and lungs. The mucus produced in the nasal passages contains a mix of antimicrobial molecules similar to those found in tears and saliva (e.g., lysozyme, lactoferrin, lactoperoxidase). Secretions in the trachea and lungs also contain lysozyme and lactoferrin, as well as a diverse group of additional chemical mediators, such as the lipoprotein complex called surfactant, which has antibacterial properties.

Note:

- Explain the difference between endogenous and exogenous mediators
- Describe how pH affects antimicrobial defenses

Antimicrobial Peptides

The **antimicrobial peptides (AMPs)** are a special class of nonspecific cell-derived mediators with broad-spectrum antimicrobial properties. Some AMPs are produced routinely by the body, whereas others are primarily produced (or produced in greater quantities) in response to the presence of an invading pathogen. Research has begun exploring how AMPs can be used in the diagnosis and treatment of disease.

AMPs may induce cell damage in microorganisms in a variety of ways, including by inflicting damage to membranes, destroying DNA and RNA, or interfering with cell-wall synthesis. Depending on the specific antimicrobial mechanism, a particular AMP may inhibit only certain groups of microbes (e.g., gram-positive or gram-negative bacteria) or it may be more broadly effective against bacteria, fungi, protozoa, and viruses. Many AMPs are found on the skin, but they can also be found in other regions of the body.

A family of AMPs called defensins can be produced by epithelial cells throughout the body as well as by cellular defenses such as macrophages and neutrophils (see [Cellular Defenses](#)). Defensins may be secreted or act inside host cells; they combat microorganisms by damaging their plasma membranes. AMPs called bacteriocins are produced exogenously by certain members of the resident microbiota within the gastrointestinal tract. The genes coding for these types of AMPs are often carried on plasmids and can be passed between different species within the resident microbiota through lateral or horizontal gene transfer.

There are numerous other AMPs throughout the body. The characteristics of a few of the more significant AMPs are summarized in [\[link\]](#).

Characteristics of Selected Antimicrobial Peptides (AMPs)				
AMP	Secreted by	Body site	Pathogens inhibited	Mode of action
Bacteriocins	Resident microbiota	Gastrointestinal tract	Bacteria	Disrupt membrane
Cathelicidin	Epithelial cells, macrophages, and other cell types	Skin	Bacteria and fungi	Disrupts membrane
Defensins	Epithelial cells, macrophages, neutrophils	Throughout the body	Fungi, bacteria, and many viruses	Disrupt membrane

Characteristics of Selected Antimicrobial Peptides (AMPs)

AMP	Secreted by	Body site	Pathogens inhibited	Mode of action
Dermicidin	Sweat glands	Skin	Bacteria and fungi	Disrupts membrane integrity and ion channels
Histatins	Salivary glands	Oral cavity	Fungi	Disrupt intracellular function

Note:

- Why are antimicrobial peptides (AMPs) considered nonspecific defenses?

Plasma Protein Mediators

Many nonspecific innate immune factors are found in **plasma**, the fluid portion of blood. Plasma contains electrolytes, sugars, lipids, and proteins, each of which helps to maintain homeostasis (i.e., stable internal body functioning), and contains the proteins involved in the clotting of blood. Additional proteins found in blood plasma, such as acute-phase proteins, complement proteins, and cytokines, are involved in the nonspecific innate immune response.

Note:

Plasma versus Serum

There are two terms for the fluid portion of blood: plasma and serum. How do they differ if they are both fluid and lack cells? The fluid portion of blood left over after coagulation (blood cell clotting) has taken place is serum. Although molecules such as many vitamins, electrolytes, certain sugars, complement proteins, and antibodies are still present in serum, clotting factors are largely depleted. Plasma, conversely, still contains all the clotting elements. To obtain plasma from blood, an anticoagulant must be used to prevent clotting. Examples of anticoagulants include heparin and ethylene diamine tetraacetic acid (EDTA).

Because clotting is inhibited, once obtained, the sample must be gently spun down in a centrifuge. The heavier, denser blood cells form a pellet at the bottom of a centrifuge tube, while the fluid plasma portion, which is lighter and less dense, remains above the cell pellet.

Acute-Phase Proteins

The **acute-phase proteins** are another class of antimicrobial mediators. Acute-phase proteins are primarily produced in the liver and secreted into the blood in response to inflammatory molecules from the immune system. Examples of acute-phase proteins include C-reactive protein, serum amyloid A, ferritin, transferrin, fibrinogen, and mannose-binding lectin. Each of these proteins has a different chemical structure and inhibits or destroys microbes in some way ([\[link\]](#)).

Some Acute-Phase Proteins and Their Functions

C-reactive protein	Coats bacteria (opsonization), preparing them for ingestion by phagocytes
Serum amyloid A	
Ferritin	Bind and sequester iron, thereby inhibiting the growth of pathogens
Transferrin	
Fibrinogen	Involved in formation of blood clots that trap bacterial pathogens
Mannose-binding lectin	Activates complement cascade

The Complement System

The **complement system** is a group of plasma protein mediators that can act as an innate nonspecific defense while also serving to connect innate and adaptive immunity (discussed in the next chapter). The complement system is composed of more than 30 proteins (including C1 through C9) that normally circulate as precursor proteins in blood. These

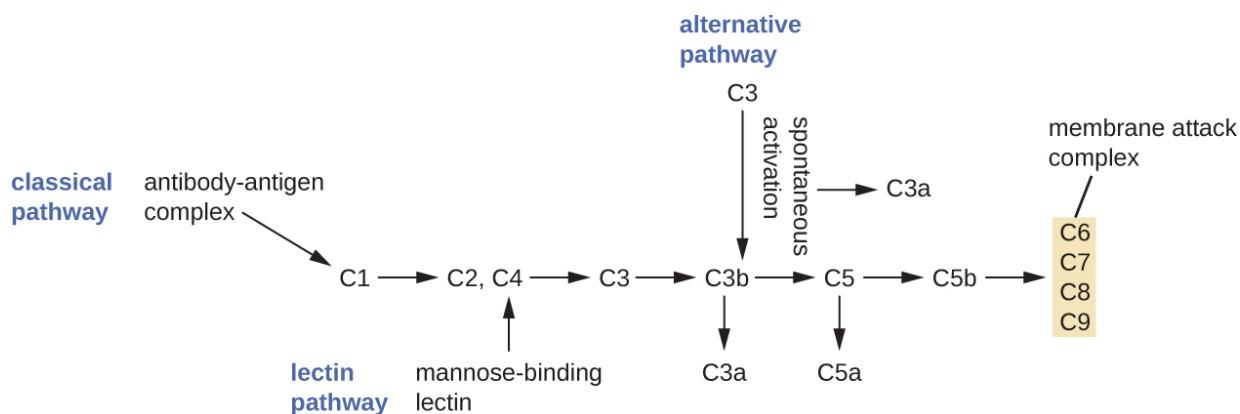
precursor proteins become activated when stimulated or triggered by a variety of factors, including the presence of microorganisms. Complement proteins are considered part of innate nonspecific immunity because they are always present in the blood and tissue fluids, allowing them to be activated quickly. Also, when activated through the alternative pathway (described later in this section), complement proteins target pathogens in a nonspecific manner.

The process by which circulating complement precursors become functional is called **complement activation**. This process is a cascade that can be triggered by one of three different mechanisms, known as the alternative, classical, and lectin pathways.

The alternative pathway is initiated by the spontaneous activation of the complement protein C3. The hydrolysis of C3 produces two products, C3a and C3b. When no invader microbes are present, C3b is very quickly degraded in a hydrolysis reaction using the water in the blood. However, if invading microbes are present, C3b attaches to the surface of these microbes. Once attached, C3b will recruit other complement proteins in a cascade ([\[link\]](#)).

The classical pathway provides a more efficient mechanism of activating the complement cascade, but it depends upon the production of antibodies by the specific adaptive immune defenses. To initiate the classical pathway, a specific antibody must first bind to the pathogen to form an antibody-antigen complex. This activates the first protein in the complement cascade, the C1 complex. The C1 complex is a multipart protein complex, and each component participates in the full activation of the overall complex. Following recruitment and activation of the C1 complex, the remaining classical pathway complement proteins are recruited and activated in a cascading sequence ([\[link\]](#)).

The lectin activation pathway is similar to the classical pathway, but it is triggered by the binding of mannose-binding lectin, an acute-phase protein, to carbohydrates on the microbial surface. Like other acute-phase proteins, lectins are produced by liver cells and are commonly upregulated in response to inflammatory signals received by the body during an infection ([\[link\]](#)).



The three complement activation pathways have different triggers, as shown here, but all three result in the activation of the complement protein C3, which produces C3a and C3b. The latter binds to the surface of the target cell and then works with other complement proteins to cleave C5 into C5a and C5b. C5b also binds to the cell surface and then recruits C6 through C9; these molecules form a ring structure called the membrane attack complex (MAC), which punches through the cell membrane of the invading pathogen, causing it to swell and burst.

Although each complement activation pathway is initiated in a different way, they all provide the same protective outcomes: opsonization, inflammation, chemotaxis, and cytolysis. The term **opsonization** refers to the coating of a pathogen by a chemical substance (called an **opsonin**) that allows phagocytic cells to recognize, engulf, and destroy it more easily. Opsonins from the complement cascade include C1q, C3b, and C4b. Additional important opsonins include mannose-binding proteins and antibodies. The complement fragments C3a and C5a are well-characterized anaphylatoxins with potent proinflammatory functions. Anaphylatoxins activate mast cells, causing degranulation and the release of inflammatory chemical signals, including mediators that cause vasodilation and increased vascular permeability. C5a is also one of the most potent chemoattractants for neutrophils and other white blood cells, cellular defenses that will be discussed in the next section.

The complement proteins C6, C7, C8, and C9 assemble into a **membrane attack complex (MAC)**, which allows C9 to polymerize into pores in the membranes of gram-negative bacteria. These pores allow water, ions, and other molecules to move freely in and out of the targeted cells, eventually leading to cell lysis and death of the pathogen ([\[link\]](#)). However, the MAC is only effective against gram-negative bacteria; it cannot penetrate the thick layer of peptidoglycan associated with cell walls of gram-positive bacteria. Since the MAC does not pose a lethal threat to gram-positive bacterial pathogens, complement-mediated opsonization is more important for their clearance.

Cytokines

Cytokines are soluble proteins that act as communication signals between cells. In a nonspecific innate immune response, various cytokines may be released to stimulate production of chemical mediators or other cell functions, such as cell proliferation, cell differentiation, inhibition of cell division, apoptosis, and chemotaxis.

When a cytokine binds to its target receptor, the effect can vary widely depending on the type of cytokine and the type of cell or receptor to which it has bound. The function of a particular cytokine can be described as autocrine, paracrine, or endocrine ([\[link\]](#)). In **autocrine function**, the same cell that releases the cytokine is the recipient of the signal; in

other words, autocrine function is a form of self-stimulation by a cell. In contrast, **paracrine function** involves the release of cytokines from one cell to other nearby cells, stimulating some response from the recipient cells. Last, **endocrine function** occurs when cells release cytokines into the bloodstream to be carried to target cells much farther away.

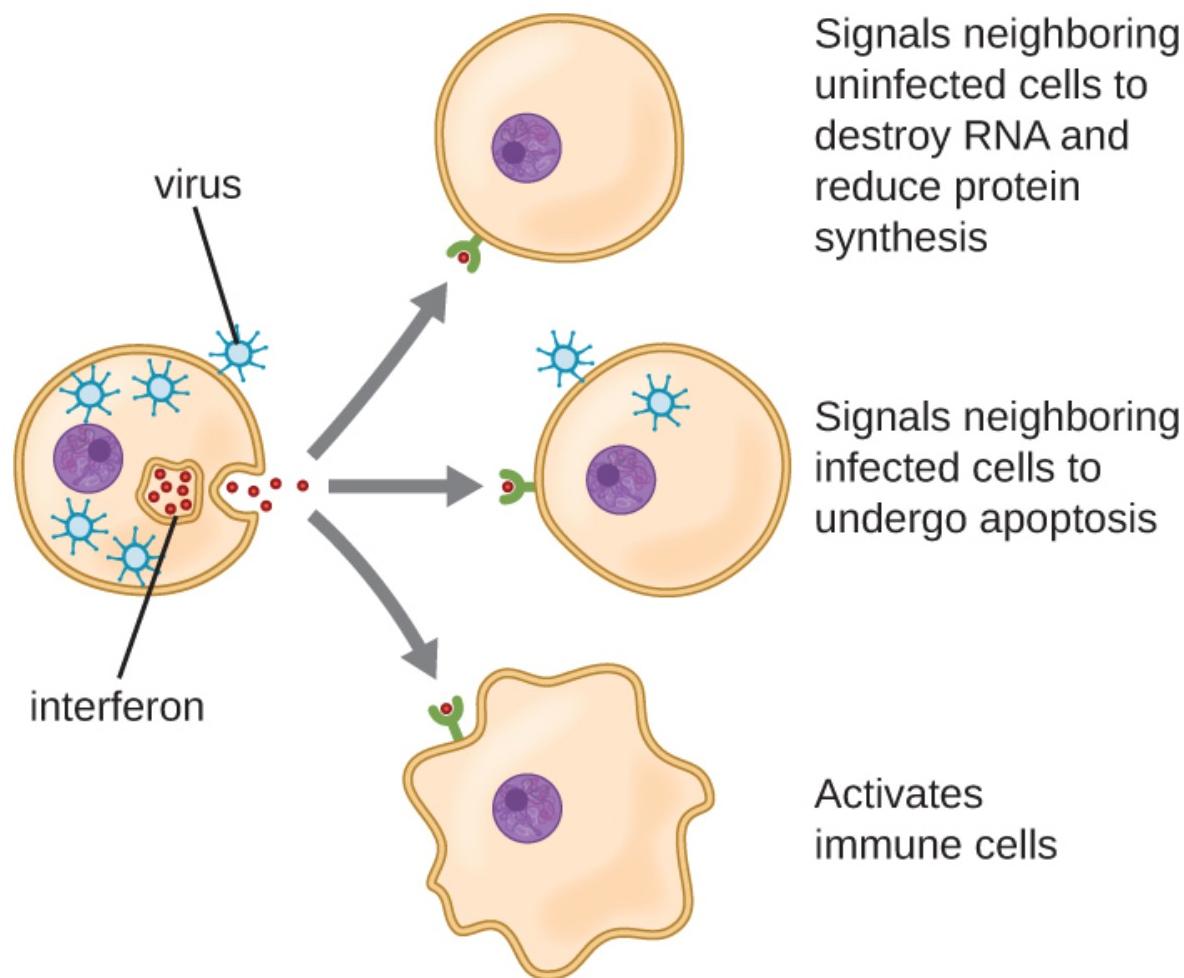
CYTOKINES: Molecular Messengers		
Autocrine	Paracrine	Endocrine
Same cell secretes and receives cytokine signal.	Cytokine signal secreted to a nearby cell.	Cytokine signal secreted to circulatory system; travels to distant cells.

Autocrine, paracrine, and endocrine actions describe which cells are targeted by cytokines and how far the cytokines must travel to bind to their intended target cells' receptors.

Three important classes of cytokines are the interleukins, chemokines, and interferons. The **interleukins** were originally thought to be produced only by leukocytes (white blood cells) and to only stimulate leukocytes, thus the reasons for their name. Although interleukins are involved in modulating almost every function of the immune system, their role in the body is not restricted to immunity. Interleukins are also produced by and stimulate a variety of cells unrelated to immune defenses.

The **chemokines** are chemotactic factors that recruit leukocytes to sites of infection, tissue damage, and inflammation. In contrast to more general chemotactic factors, like complement factor C5a, chemokines are very specific in the subsets of leukocytes they recruit.

Interferons are a diverse group of immune signaling molecules and are especially important in our defense against viruses. Type I **interferons** (interferon- α and interferon- β) are produced and released by cells infected with virus. These interferons stimulate nearby cells to stop production of mRNA, destroy RNA already produced, and reduce protein synthesis. These cellular changes inhibit viral replication and production of mature virus, slowing the spread of the virus. Type I interferons also stimulate various immune cells involved in viral clearance to more aggressively attack virus-infected cells. Type II interferon (interferon- γ) is an important activator of immune cells ([\[link\]](#)).



Interferons are cytokines released by a cell infected with a virus. Interferon- α and interferon- β signal uninfected neighboring cells to inhibit mRNA synthesis, destroy RNA, and reduce protein synthesis (top arrow). Interferon- α and interferon- β also promote apoptosis in cells infected with the virus (middle arrow). Interferon- γ alerts neighboring immune cells to an attack (bottom arrow). Although interferons do not cure the cell releasing them or other infected cells,

which will soon die, their release may prevent additional cells from becoming infected, thus stemming the infection.

Inflammation-Eliciting Mediators

Many of the chemical mediators discussed in this section contribute in some way to inflammation and fever, which are nonspecific immune responses discussed in more detail in [Inflammation and Fever](#). Cytokines stimulate the production of acute-phase proteins such as C-reactive protein and mannose-binding lectin in the liver. These acute-phase proteins act as opsonins, activating complement cascades through the lectin pathway.

Some cytokines also bind mast cells and basophils, inducing them to release **histamine**, a proinflammatory compound. Histamine receptors are found on a variety of cells and mediate proinflammatory events, such as bronchoconstriction (tightening of the airways) and smooth muscle contraction.

In addition to histamine, mast cells may release other chemical mediators, such as **leukotrienes**. Leukotrienes are lipid-based proinflammatory mediators that are produced from the metabolism of arachidonic acid in the cell membrane of leukocytes and tissue cells. Compared with the proinflammatory effects of histamine, those of leukotrienes are more potent and longer lasting. Together, these chemical mediators can induce coughing, vomiting, and diarrhea, which serve to expel pathogens from the body.

Certain cytokines also stimulate the production of prostaglandins, chemical mediators that promote the inflammatory effects of kinins and histamines. Prostaglandins can also help to set the body temperature higher, leading to fever, which promotes the activities of white blood cells and slightly inhibits the growth of pathogenic microbes (see [Inflammation and Fever](#)).

Another inflammatory mediator, **bradykinin**, contributes to edema, which occurs when fluids and leukocytes leak out of the bloodstream and into tissues. It binds to receptors on cells in the capillary walls, causing the capillaries to dilate and become more permeable to fluids.

Note:

- What do the three complement activation pathways have in common?
- Explain autocrine, paracrine, and endocrine signals.
- Name two important inflammation-eliciting mediators.

[\[link\]](#) provides a summary of the chemical defenses discussed in this section.

Chemical Defenses of Nonspecific Innate Immunity		
Defense	Examples	Function
Chemicals and enzymes in body fluids	Sebum from sebaceous glands	Provides oil barrier protecting hair follicle pores from pathogens
	Oleic acid from sebum and skin microbiota	Lowers pH to inhibit pathogens
	Lysozyme in secretions	Kills bacteria by attacking cell wall
	Acid in stomach, urine, and vagina	Inhibits or kills bacteria
	Digestive enzymes and bile	Kill bacteria
	Lactoferrin and transferrin	Bind and sequester iron, inhibiting bacterial growth
	Surfactant in lungs	Kills bacteria
Antimicrobial peptides	Defensins, bacteriocins, dermicidin, cathelicidin, histatins,	Kill bacteria by attacking membranes or interfering with cell functions
Plasma protein mediators	Acute-phase proteins (C-reactive protein, serum amyloid A, ferritin, fibrinogen, transferrin, and mannose-binding lectin)	Inhibit the growth of bacteria and assist in the trapping and killing of bacteria

Chemical Defenses of Nonspecific Innate Immunity		
Defense	Examples	Function
Cytokines	Complements C3b and C4b	Opsonization of pathogens to aid phagocytosis
	Complement C5a	Chemoattractant for phagocytes
	Complements C3a and C5a	Proinflammatory anaphylatoxins
Cytokines	Interleukins	Stimulate and modulate most functions of immune system
	Chemokines	Recruit white blood cells to infected area
	Interferons	Alert cells to viral infection, induce apoptosis of virus-infected cells, induce antiviral defenses in infected and nearby uninfected cells, stimulate immune cells to attack virus-infected cells
Inflammation-eliciting mediators	Histamine	Promotes vasodilation, bronchoconstriction, smooth muscle contraction, increased secretion and mucus production
	Leukotrienes	Promote inflammation; stronger and longer lasting than histamine
	Prostaglandins	Promote inflammation and fever
	Bradykinin	Increases vasodilation and vascular permeability, leading to edema

Key Concepts and Summary

- Numerous **chemical mediators** produced endogenously and exogenously exhibit nonspecific antimicrobial functions.

- Many chemical mediators are found in body fluids such as sebum, saliva, mucus, gastric and intestinal fluids, urine, tears, cerumen, and vaginal secretions.
- **Antimicrobial peptides (AMPs)** found on the skin and in other areas of the body are largely produced in response to the presence of pathogens. These include dermcidin, cathelicidin, defensins, histatins, and bacteriocins.
- **Plasma** contains various proteins that serve as chemical mediators, including **acute-phase proteins, complement proteins, and cytokines**.
- The **complement system** involves numerous precursor proteins that circulate in plasma. These proteins become activated in a cascading sequence in the presence of microbes, resulting in the **opsonization** of pathogens, chemoattraction of leukocytes, induction of inflammation, and cytolysis through the formation of a **membrane attack complex (MAC)**.
- **Cytokines** are proteins that facilitate various nonspecific responses by innate immune cells, including production of other chemical mediators, cell proliferation, cell death, and differentiation.
- Cytokines play a key role in the inflammatory response, triggering production of inflammation-eliciting mediators such as acute-phase proteins, **histamine**, leukotrienes, **prostaglandins**, and **bradykinin**.

Short Answer

Exercise:

Problem:

Differentiate the main activation methods of the classic, alternative, and lectin complement cascades.

Exercise:

Problem: What are the four protective outcomes of complement activation?

Cellular Defenses

LEARNING OBJECTIVES

- Identify and describe the components of blood
- Explain the process by which the formed elements of blood are formed (hematopoiesis)
- Describe the characteristics of formed elements found in peripheral blood, as well as their respective functions within the innate immune system

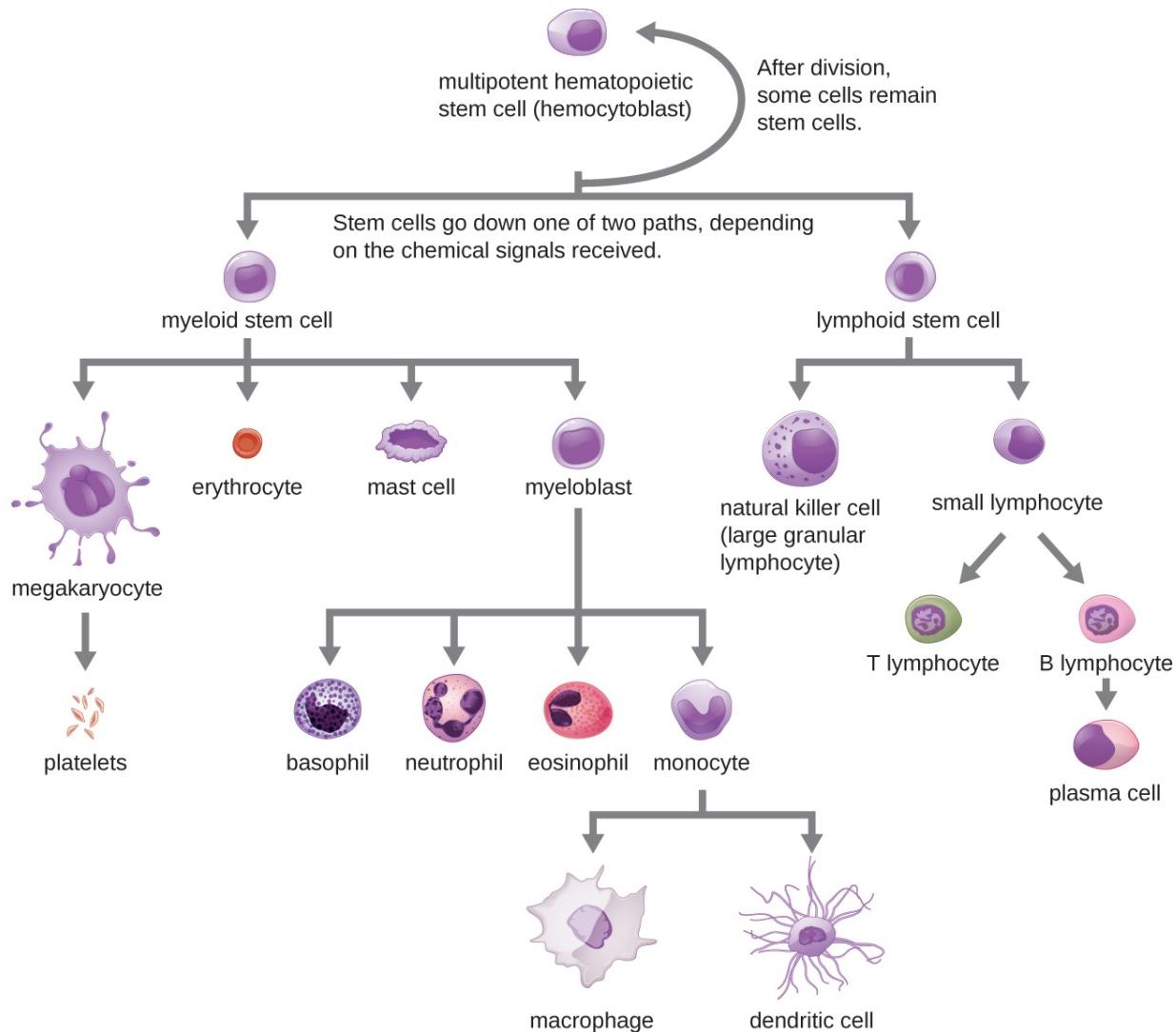
In the previous section, we discussed some of the chemical mediators found in plasma, the fluid portion of blood. The nonfluid portion of blood consists of various types of formed elements, so called because they are all formed from the same stem cells found in bone marrow. The three major categories of formed elements are: red blood cells (RBCs), also called **erythrocytes**; **platelets**, also called **thrombocytes**; and white blood cells (WBCs), also called **leukocytes**.

Red blood cells are primarily responsible for carrying oxygen to tissues. Platelets are cellular fragments that participate in blood clot formation and tissue repair. Several different types of WBCs participate in various nonspecific mechanisms of innate and adaptive immunity. In this section, we will focus primarily on the innate mechanisms of various types of WBCs.

Hematopoiesis

All of the formed elements of blood are derived from pluripotent hematopoietic stem cells (HSCs) in the bone marrow. As the HSCs make copies of themselves in the bone marrow, individual cells receive different cues from the body that control how they develop and mature. As a result, the HSCs differentiate into different types of blood cells that, once mature, circulate in peripheral blood. This process of differentiation, called **hematopoiesis**, is shown in more detail in [\[link\]](#).

In terms of sheer numbers, the vast majority of HSCs become erythrocytes. Much smaller numbers become leukocytes and platelets. Leukocytes can be further subdivided into **granulocytes**, which are characterized by numerous granules visible in the cytoplasm, and agranulocytes, which lack granules. [\[link\]](#) provides an overview of the various types of formed elements, including their relative numbers, primary function, and lifespans.



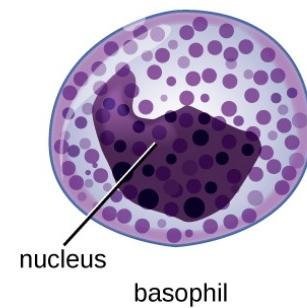
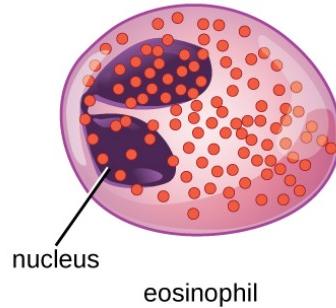
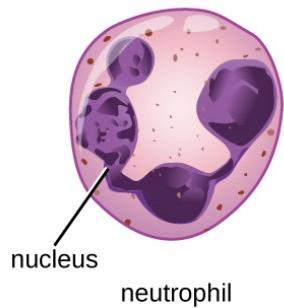
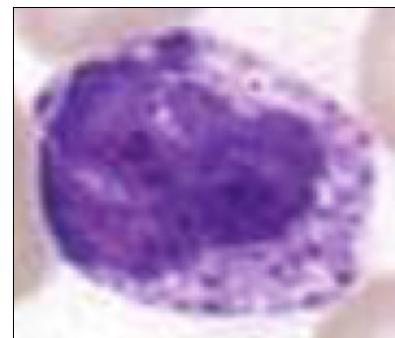
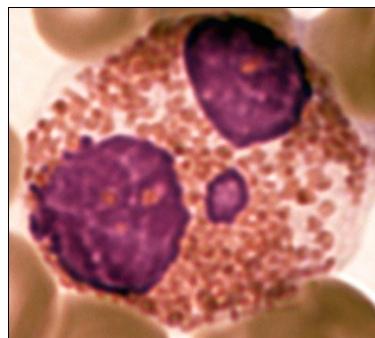
All the formed elements of the blood arise by differentiation of hematopoietic stem cells in the bone marrow.

Formed Element	Major Subtypes		Numbers Present per Microliter (μL) and Mean (Range)	Appearance in a Standard Blood Smear	Summary of Functions	Comments
Erythrocytes <i>(red blood cells)</i>			5.2 million (4.4–6.0 million)	Flattened biconcave disk; no nucleus; pale red	Transport oxygen and some carbon dioxide between tissue and lungs	Lifespan of approximately 120 days
Leukocytes <i>(white blood cells)</i>			7000 (5000–10,000)	Obvious dark-staining nucleus	All function in body defenses	Exit capillaries and move into tissues; lifespan of usually a few hours or days
Granulocytes, <i>including neutrophils, eosinophils, and basophils</i>		Total leukocytes (%)	4360 (1800–9950)	Abundant granules in cytoplasm; nucleus normally lobed	Nonspecific (innate) resistance to disease	Classified according to membrane-bound granules in cytoplasm
Neutrophils		50–70	4150 (1800–7300)	Nucleus lobes increase with age; pale lilac granules	Phagocytic; particularly effective against bacteria; release cytotoxic chemicals from granules	Most common leukocyte; lifespan of minutes to days
Eosinophils		1–3	165 (0–700)	Nucleus generally two-lobed; bright red-orange granules	Phagocytic cells; particularly effective with antigen-antibody complexes; release antihistamines; combat parasitic infections	Lifespan of minutes to days
Basophils		<1	44 (0–150)	Nucleus generally two-lobed but difficult to see due to presence of heavy, dense, dark purple granules	Pro-inflammatory	Least common leukocyte; lifespan unknown
Agranulocytes, <i>including lymphocytes and monocytes</i>			2640 (1700–4950)	Lack abundant granules in cytoplasm; have a simple-shaped nucleus that may be indented	Body defenses	Group consists of two major cell types from different lineages
Lymphocytes	 	20–40	2185 (1500–4000)	Spherical cells with a single, often large, nucleus occupying much of the cell's volume; stains purple; seen in large (natural killer cells) and small (B and T cells) variants	Primarily specific (adaptive) immunity: T cells directly attack other cells (cellular immunity); B cells release antibodies (humoral immunity); natural killer cells are similar to T cells but nonspecific	Initial cells originate in bone marrow, but secondary production occurs in lymphatic tissue; several distinct subtypes; memory cells form after exposure to a pathogen and rapidly increase responses to subsequent exposure; lifespan of many years
Monocytes		1–6	455 (200–950)	Largest leukocyte; has an indented or horseshoe-shaped nucleus	Very effective phagocytic cells engulfing pathogens or worn-out cells; also serve as antigen-presenting cells (APCs) or other components of the immune system	Produced in red bone marrow; referred to as macrophages and dendritic cells after leaving the circulation
Platelets			350,000 (150,000–500,000)	Cellular fragments surrounded by a plasma membrane and containing granules; stains purple	Hemostasis; release growth factors for repair and healing of tissue	Formed from megakaryocytes that remain in the red bone marrow and shed platelets into circulation

Formed elements of blood include erythrocytes (red blood cells), leukocytes (white blood cells), and platelets.

Granulocytes

The various types of granulocytes can be distinguished from one another in a blood smear by the appearance of their nuclei and the contents of their granules, which confer different traits, functions, and staining properties. The **neutrophils**, also called **polymorphonuclear neutrophils (PMNs)**, have a nucleus with three to five lobes and small, numerous, lilac-colored granules. Each lobe of the nucleus is connected by a thin strand of material to the other lobes. The **eosinophils** have fewer lobes in the nucleus (typically 2–3) and larger granules that stain reddish-orange. The **basophils** have a two-lobed nucleus and large granules that stain dark blue or purple ([\[link\]](#)).



Granulocytes can be distinguished by the number of lobes in their nuclei and the staining properties of their granules. (credit “neutrophil” micrograph: modification of work by Ed Uthman)

Neutrophils (PMNs)

Neutrophils (PMNs) are frequently involved in the elimination and destruction of extracellular bacteria. They are capable of migrating through the walls of blood vessels to areas of bacterial infection and tissue damage, where they seek out and kill infectious bacteria. PMN granules contain a variety of defensins and hydrolytic enzymes that help them destroy bacteria through phagocytosis (described in more detail in [Pathogen Recognition and Phagocytosis](#)) In addition, when many neutrophils are brought into an infected area, they can be stimulated to release toxic molecules into the surrounding tissue to better clear infectious agents. This is called degranulation.

Another mechanism used by neutrophils is neutrophil extracellular traps (NETs), which are extruded meshes of chromatin that are closely associated with antimicrobial granule proteins and components. Chromatin is DNA with associated proteins (usually histone proteins, around which DNA wraps for organization and packing within a cell). By creating and releasing a mesh or lattice-like structure of chromatin that is coupled with antimicrobial proteins, the neutrophils can mount a highly concentrated and efficient attack against nearby pathogens. Proteins frequently associated with NETs include lactoferrin, gelatinase, cathepsin G, and myeloperoxidase. Each has a different means of promoting antimicrobial activity, helping neutrophils eliminate pathogens. The toxic proteins in NETs may kill some of the body's own cells along with invading pathogens. However, this collateral damage can be repaired after the danger of the infection has been eliminated.

As neutrophils fight an infection, a visible accumulation of leukocytes, cellular debris, and bacteria at the site of infection can be observed. This buildup is what we call **pus** (also known as purulent or suppurative discharge or drainage). The presence of pus is a sign that the immune defenses have been activated against an infection; historically, some physicians believed that inducing pus formation could actually promote the healing of wounds. The practice of promoting “laudable pus” (by, for

instance, wrapping a wound in greasy wool soaked in wine) dates back to the ancient physician Galen in the 2nd century AD, and was practiced in variant forms until the 17th century (though it was not universally accepted). Today, this method is no longer practiced because we now know that it is not effective. Although a small amount of pus formation can indicate a strong immune response, artificially inducing pus formation does not promote recovery.

Eosinophils

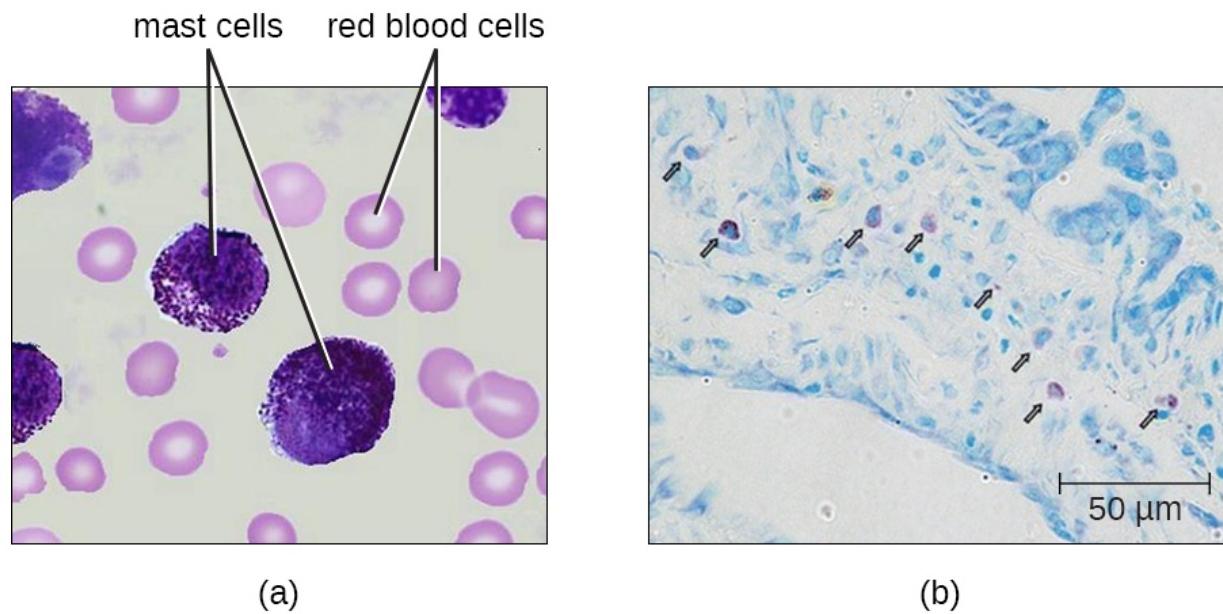
Eosinophils are granulocytes that protect against protozoa and helminths; they also play a role in allergic reactions. The granules of eosinophils, which readily absorb the acidic reddish dye eosin, contain histamine, degradative enzymes, and a compound known as major basic protein (MBP) ([\[link\]](#)). MBP binds to the surface carbohydrates of parasites, and this binding is associated with disruption of the cell membrane and membrane permeability.

Basophils

Basophils have cytoplasmic granules of varied size and are named for their granules' ability to absorb the basic dye methylene blue ([\[link\]](#)). Their stimulation and degranulation can result from multiple triggering events. Activated complement fragments C3a and C5a, produced in the activation cascades of complement proteins, act as anaphylatoxins by inducing degranulation of basophils and inflammatory responses. This cell type is important in allergic reactions and other responses that involve inflammation. One of the most abundant components of basophil granules is histamine, which is released along with other chemical factors when the basophil is stimulated. These chemicals can be chemotactic and can help to open the gaps between cells in the blood vessels. Other mechanisms for basophil triggering require the assistance of antibodies, as discussed in [B Lymphocytes and Humoral Immunity](#).

Mast Cells

Hematopoiesis also gives rise to **mast cells**, which appear to be derived from the same common myeloid progenitor cell as neutrophils, eosinophils, and basophils. Functionally, mast cells are very similar to basophils, containing many of the same components in their granules (e.g., histamine) and playing a similar role in allergic responses and other inflammatory reactions. However, unlike basophils, mast cells leave the circulating blood and are most frequently found residing in tissues. They are often associated with blood vessels and nerves or found close to surfaces that interface with the external environment, such as the skin and mucous membranes in various regions of the body ([\[link\]](#)).



Mast cells function similarly to basophils by inducing and promoting inflammatory responses. (a) This figure shows mast cells in blood. In a blood smear, they are difficult to differentiate from basophils (b). Unlike basophils, mast cells migrate from the blood into various tissues. (credit right: modification of work by Greenland JR, Xu X, Sayah DM, Liu FC, Jones KD, Looney MR, Caughey GH)

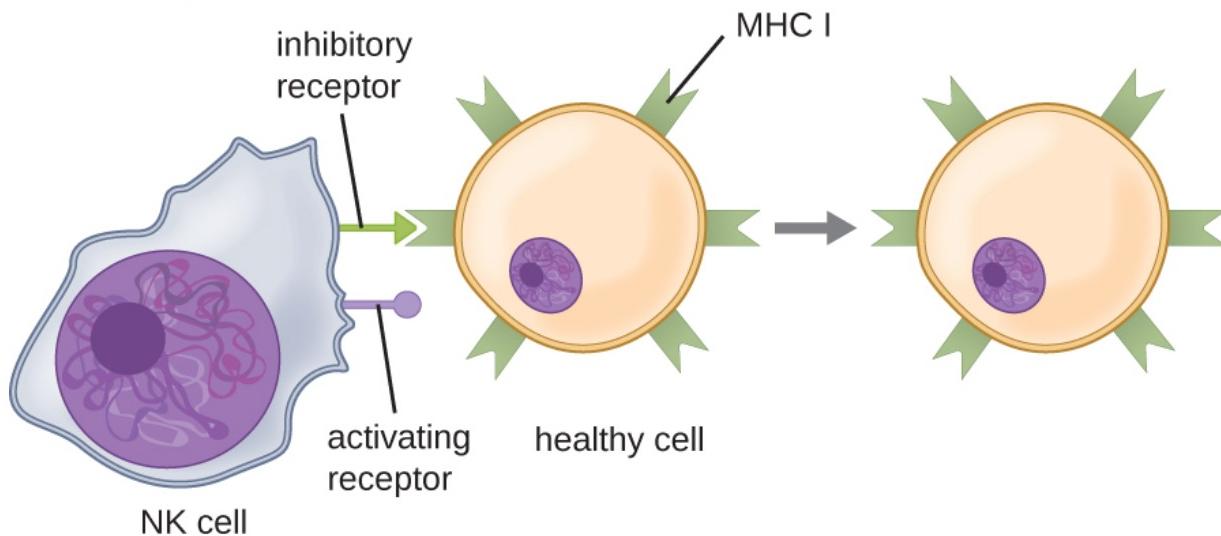
Agranulocytes

As their name suggests, **agranulocytes** lack visible granules in the cytoplasm. Agranulocytes can be categorized as lymphocytes or monocytes ([\[link\]](#)). Among the lymphocytes are natural killer cells, which play an important role in nonspecific innate immune defenses. Lymphocytes also include the B cells and T cells, which are discussed in the next chapter because they are central players in the specific adaptive immune defenses. The monocytes differentiate into macrophages and dendritic cells, which are collectively referred to as the mononuclear phagocyte system.

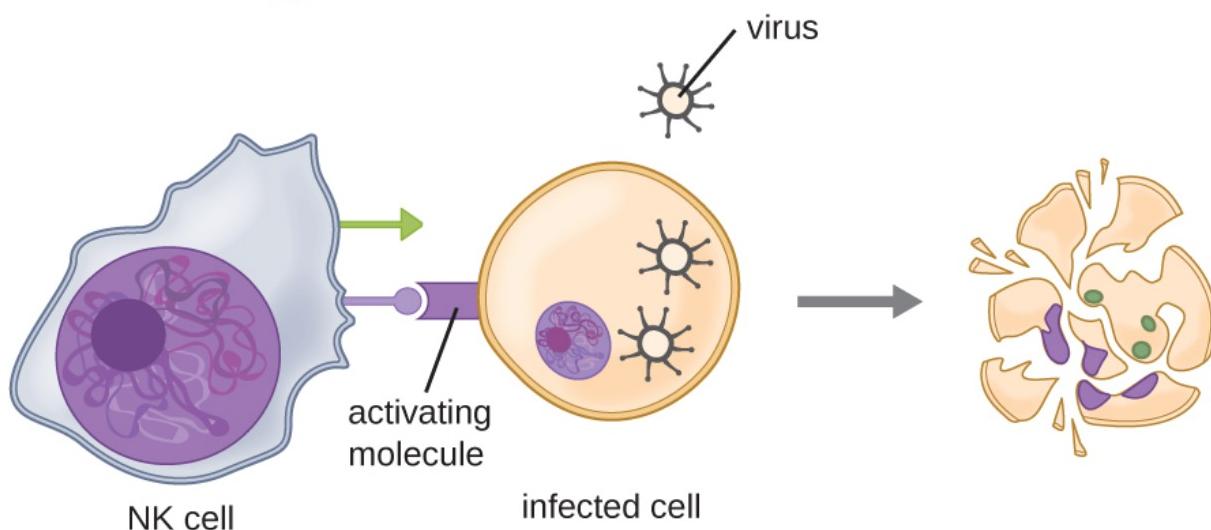
Natural Killer Cells

Most lymphocytes are primarily involved in the specific adaptive immune response, and thus will be discussed in the following chapter. An exception is the **natural killer cells (NK cells)**; these mononuclear lymphocytes use nonspecific mechanisms to recognize and destroy cells that are abnormal in some way. Cancer cells and cells infected with viruses are two examples of cellular abnormalities that are targeted by NK cells. Recognition of such cells involves a complex process of identifying inhibitory and activating molecular markers on the surface of the target cell. Molecular markers that make up the major histocompatibility complex (MHC) are expressed by healthy cells as an indication of “self.” This will be covered in more detail in next chapter. NK cells are able to recognize normal MHC markers on the surface of healthy cells, and these MHC markers serve as an inhibitory signal preventing NK cell activation. However, cancer cells and virus-infected cells actively diminish or eliminate expression of MHC markers on their surface. When these MHC markers are diminished or absent, the NK cell interprets this as an abnormality and a cell in distress. This is one part of the NK cell activation process ([\[link\]](#)). NK cells are also activated by binding to activating molecular molecules on the target cell. These activating molecular molecules include “altered self” or “nonself” molecules. When a NK cell recognizes a decrease in inhibitory normal MHC molecules and an increase in activating molecules on the surface of a cell, the NK cell will be activated to eliminate the cell in distress.

A natural killer (NK) cell recognizes MHC I on a healthy cell and does not kill it.



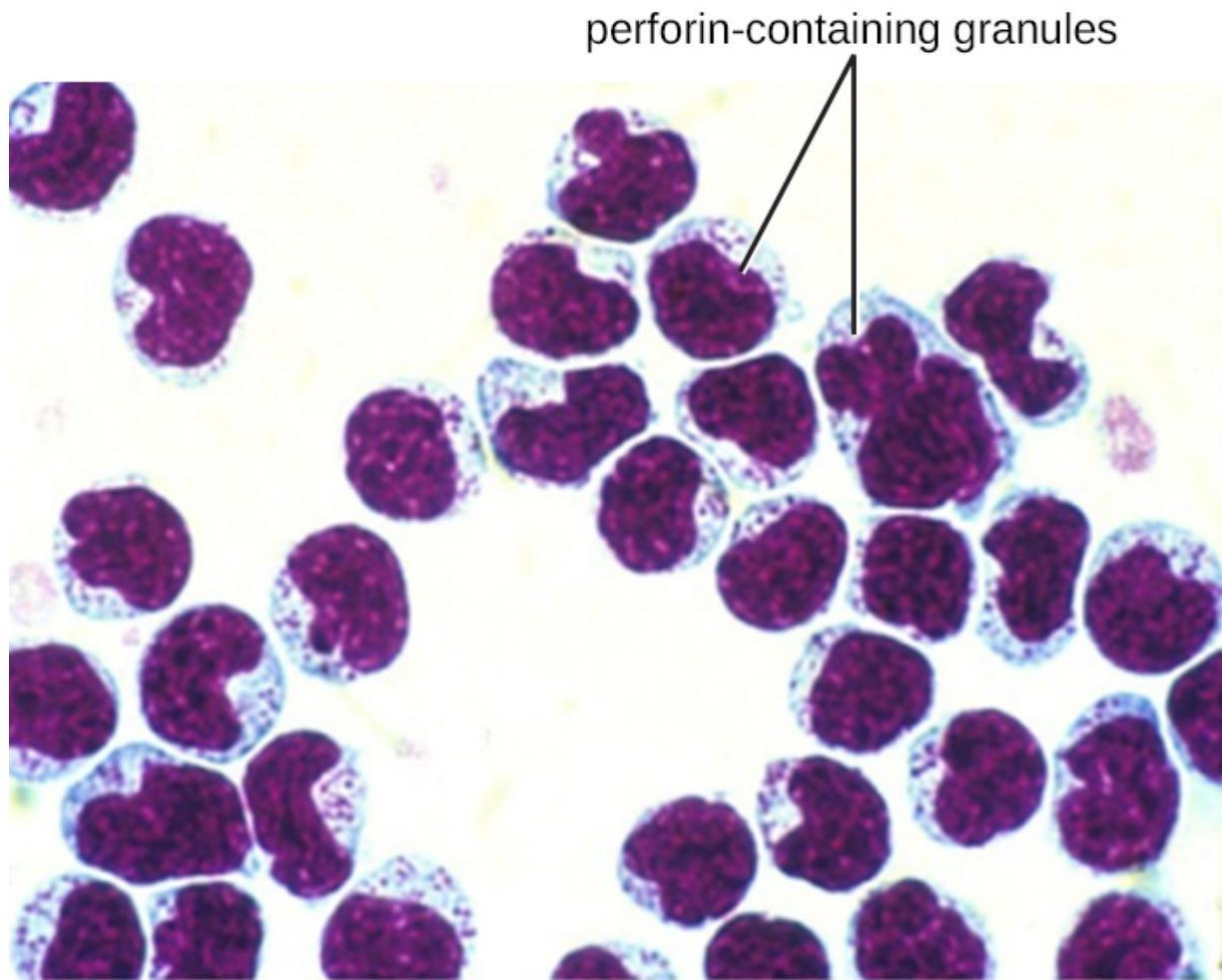
An infected cell does not present the MHC I, but does present ligands for the activating receptor. The NK cell will trigger a response that kills this cell.



Natural killer (NK) cells are inhibited by the presence of the major histocompatibility cell (MHC) receptor on healthy cells. Cancer cells and virus-infected cells have reduced expression of MHC and increased expression of activating molecules. When a NK cell recognizes decreased MHC and increased activating molecules, it will kill the abnormal cell.

Once a cell has been recognized as a target, the NK cell can use several different mechanisms to kill its target. For example, it may express cytotoxic membrane proteins and cytokines that stimulate the target cell to undergo apoptosis, or controlled cell suicide. NK cells may also use perforin-mediated cytotoxicity to induce apoptosis in target cells. This mechanism relies on two toxins released from granules in the cytoplasm of the NK cell: **perforin**, a protein that creates pores in the target cell, and **granzymes**, proteases that enter through the pores into the target cell's cytoplasm, where they trigger a cascade of protein activation that leads to apoptosis. The NK cell binds to the abnormal target cell, releases its destructive payload, and detaches from the target cell. While the target cell undergoes apoptosis, the NK cell synthesizes more perforin and proteases to use on its next target.

NK cells contain these toxic compounds in granules in their cytoplasm. When stained, the granules are azurophilic and can be visualized under a light microscope ([\[link\]](#)). Even though they have granules, NK cells are not considered granulocytes because their granules are far less numerous than those found in true granulocytes. Furthermore, NK cells have a different lineage than granulocytes, arising from lymphoid rather than myeloid stem cells ([\[link\]](#)).

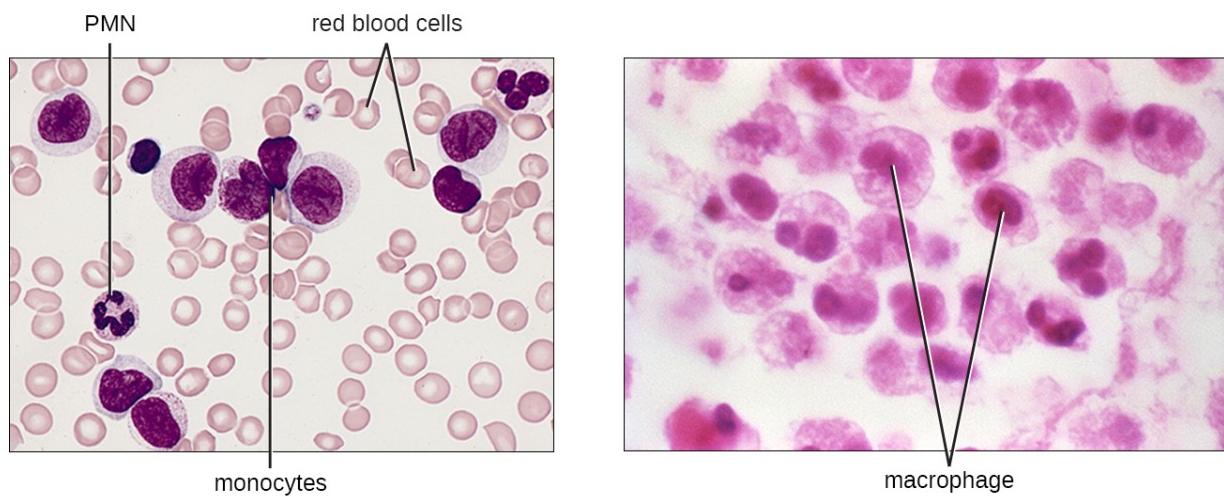


Natural killer cell with perforin-containing granules. (credit: modification of work by Rolstad B)

Monocytes

The largest of the white blood cells, **monocytes** have a nucleus that lacks lobes, and they also lack granules in the cytoplasm ([\[link\]](#)). Nevertheless, they are effective phagocytes, engulfing pathogens and apoptotic cells to help fight infection.

When monocytes leave the bloodstream and enter a specific body tissue, they differentiate into tissue-specific phagocytes called **macrophages** and **dendritic cells**. They are particularly important residents of lymphoid tissue, as well as nonlymphoid sites and organs. Macrophages and dendritic cells can reside in body tissues for significant lengths of time. Macrophages in specific body tissues develop characteristics suited to the particular tissue. Not only do they provide immune protection for the tissue in which they reside but they also support normal function of their neighboring tissue cells through the production of cytokines. Macrophages are given tissue-specific names, and a few examples of tissue-specific macrophages are listed in [link]. Dendritic cells are important sentinels residing in the skin and mucous membranes, which are portals of entry for many pathogens. Monocytes, macrophages, and dendritic cells are all highly phagocytic and important promoters of the immune response through their production and release of cytokines. These cells provide an essential bridge between innate and adaptive immune responses, as discussed in the next section as well as the next chapter.



Monocytes are large, agranular white blood cells with a nucleus that lacks lobes. When monocytes leave the bloodstream, they differentiate and become macrophages with tissue-specific properties. (credit left: modification of work by Armed Forces Institute of Pathology; credit right: modification of work by Centers for Disease Control and Prevention)

Macrophages Found in Various Body Tissues

Tissue	Macrophage
Brain and central nervous system	Microglial cells
Liver	Kupffer cells
Lungs	Alveolar macrophages (dust cells)
Peritoneal cavity	Peritoneal macrophages

Note:

- Describe the signals that activate natural killer cells.
- What is the difference between monocytes and macrophages?

Key Concepts and Summary

- The **formed elements** of the blood include red blood cells (**erythrocytes**), white blood cells (**leukocytes**), and **platelets**

(thrombocytes). Of these, leukocytes are primarily involved in the immune response.

- All formed elements originate in the bone marrow as stem cells (HSCs) that differentiate through **hematopoiesis**.
- **Granulocytes** are leukocytes characterized by a lobed nucleus and granules in the cytoplasm. These include **neutrophils (PMNs)**, **eosinophils**, and **basophils**.
- Neutrophils are the leukocytes found in the largest numbers in the bloodstream and they primarily fight bacterial infections.
- Eosinophils target parasitic infections. Eosinophils and basophils are involved in allergic reactions. Both release histamine and other proinflammatory compounds from their granules upon stimulation.
- **Mast cells** function similarly to basophils but can be found in tissues outside the bloodstream.
- **Natural killer (NK)** cells are lymphocytes that recognize and kill abnormal or infected cells by releasing proteins that trigger apoptosis.
- **Monocytes** are large, mononuclear leukocytes that circulate in the bloodstream. They may leave the bloodstream and take up residence in body tissues, where they differentiate and become tissue-specific **macrophages** and **dendritic cells**.

Short Answer

Exercise:

Problem:

Explain the difference between plasma and the formed elements of the blood.

Exercise:

Problem:

List three ways that a neutrophil can destroy an infectious bacterium.

Critical Thinking

Exercise:

Problem:

Neutrophils can sometimes kill human cells along with pathogens when they release the toxic contents of their granules into the surrounding tissue. Likewise, natural killer cells target human cells for destruction. Explain why it is advantageous for the immune system to have cells that can kill human cells as well as pathogens.

Pathogen Recognition and Phagocytosis

LEARNING OBJECTIVES

- Explain how leukocytes migrate from peripheral blood into infected tissues
- Explain the mechanisms by which leukocytes recognize pathogens
- Explain the process of phagocytosis and the mechanisms by which phagocytes destroy and degrade pathogens

Several of the cell types discussed in the previous section can be described as phagocytes—cells whose main function is to seek, ingest, and kill pathogens. This process, called phagocytosis, was first observed in starfish in the 1880s by Nobel Prize-winning zoologist Ilya Metchnikoff (1845–1916), who made the connection to white blood cells (WBCs) in humans and other animals. At the time, Pasteur and other scientists believed that WBCs were spreading pathogens rather than killing them (which is true for some diseases, such as tuberculosis). But in most cases, phagocytes provide a strong, swift, and effective defense against a broad range of microbes, making them a critical component of innate nonspecific immunity. This section will focus on the mechanisms by which phagocytes are able to seek, recognize, and destroy pathogens.

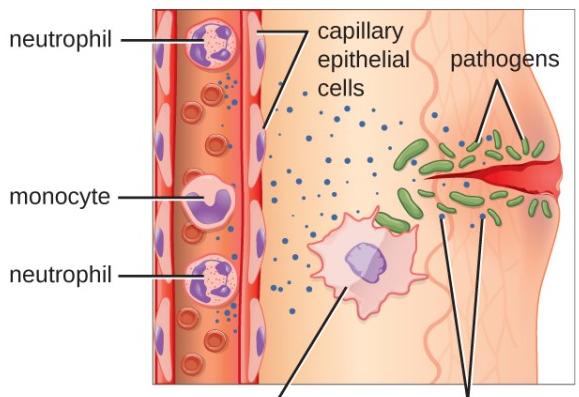
Extravasation (Diapedesis) of Leukocytes

Some phagocytes are leukocytes (WBCs) that normally circulate in the bloodstream. To reach pathogens located in infected tissue, leukocytes must pass through the walls of small capillary blood vessels within tissues. This

process, called **extravasation**, or **diapedesis**, is initiated by complement factor C5a, as well as cytokines released into the immediate vicinity by resident macrophages and tissue cells responding to the presence of the infectious agent ([\[link\]](#)). Similar to C5a, many of these cytokines are proinflammatory and chemotactic, and they bind to cells of small capillary blood vessels, initiating a response in the endothelial cells lining the inside of the blood vessel walls. This response involves the upregulation and expression of various cellular adhesion molecules and receptors.

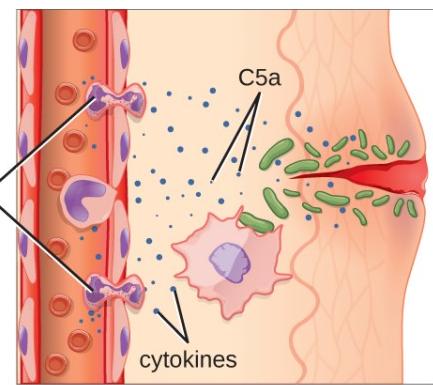
Leukocytes passing through will stick slightly to the adhesion molecules, slowing down and rolling along the blood vessel walls near the infected area. When they reach a cellular junction, they will bind to even more of these adhesion molecules, flattening out and squeezing through the cellular junction in a process known as **transendothelial migration**. This mechanism of “rolling adhesion” allows leukocytes to exit the bloodstream and enter the infected areas, where they can begin phagocytosing the invading pathogens.

Note that extravasation does not occur in arteries or veins. These blood vessels are surrounded by thicker, multilayer protective walls, in contrast to the thin single-cell-layer walls of capillaries. Furthermore, the blood flow in arteries is too turbulent to allow for rolling adhesion. Also, some leukocytes tend to respond to an infection more quickly than others. The first to arrive typically are neutrophils, often within hours of a bacterial infection. By contrast, monocytes may take several days to leave the bloodstream and differentiate into macrophages.



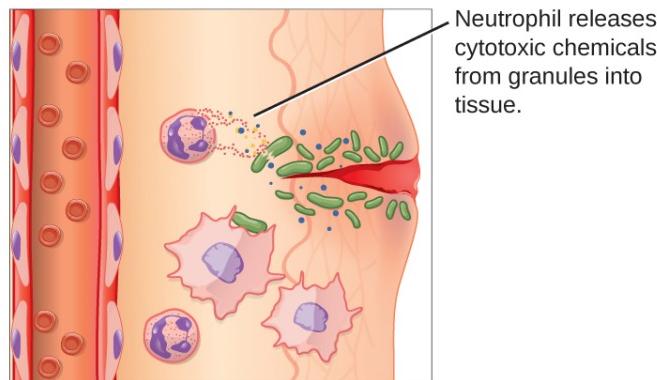
Resident macrophage engulfs pathogen and releases proinflammatory, chemotactic cytokines.
Injured/infected cells secrete chemical signals into the blood.

- Leukocytes in the blood respond to chemical attractants released by pathogens and chemical signals from nearby injured cells.



Leukocytes emigrate to site of injury and infection.

- The leukocytes squeeze between the cells of the capillary wall as they follow the chemical signals to where they are most concentrated (positive chemotaxis).



- Within the damaged tissue, neutrophils release chemicals that break apart pathogens. Monocytes differentiate into macrophages. Neutrophils and macrophages phagocytize pathogens and cellular debris.

Damaged cells and macrophages that have ingested pathogens release cytokines that are proinflammatory and chemotactic for leukocytes. In addition, activation of complement at the site of infection results in production of the chemotactic and proinflammatory C5a. Leukocytes exit the blood vessel and follow the chemoattractant signal of cytokines and C5a to the site of infection. Granulocytes such as neutrophils release chemicals that destroy pathogens. They are also capable of phagocytosis and intracellular killing of bacterial pathogens.

Note:



Watch the following videos on [leukocyte extravasation](#) and [leukocyte rolling](#) to learn more.

Note:

- Explain the role of adhesion molecules in the process of extravasation.

Pathogen Recognition

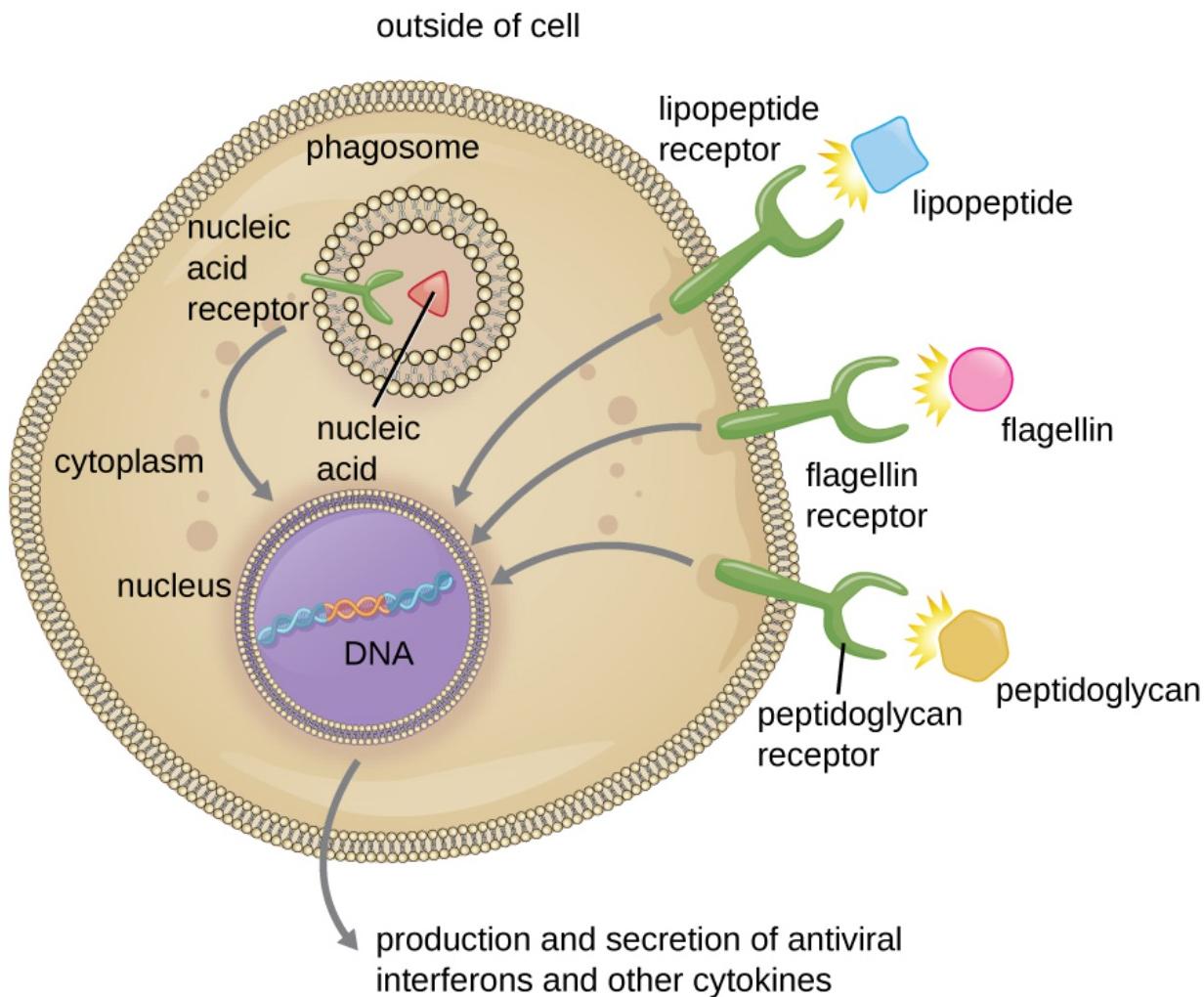
As described in the previous section, opsonization of pathogens by antibody; complement factors C1q, C3b, and C4b; and lectins can assist phagocytic cells in recognition of pathogens and attachment to initiate phagocytosis. However, not all pathogen recognition is opsonin dependent. Phagocytes can also recognize molecular structures that are common to many groups of pathogenic microbes. Such structures are called **pathogen-associated molecular patterns (PAMPs)**. Common PAMPs include the following:

- peptidoglycan, found in bacterial cell walls;
- flagellin, a protein found in bacterial flagella;
- lipopolysaccharide (LPS) from the outer membrane of gram-negative bacteria;
- lipopeptides, molecules expressed by most bacteria; and
- nucleic acids such as viral DNA or RNA.

Like numerous other PAMPs, these substances are integral to the structure of broad classes of microbes.

The structures that allow phagocytic cells to detect PAMPs are called **pattern recognition receptors (PRRs)**. One group of PRRs is the **toll-like receptors (TLRs)**, which bind to various PAMPs and communicate with the nucleus of the phagocyte to elicit a response. Many TLRs (and other PRRs) are located on the surface of a phagocyte, but some can also be found embedded in the membranes of interior compartments and organelles ([\[link\]](#)). These interior PRRs can be useful for the binding and recognition of intracellular pathogens that may have gained access to the inside of the cell before phagocytosis could take place. Viral nucleic acids, for example, might encounter an interior PRR, triggering production of the antiviral cytokine interferon.

In addition to providing the first step of pathogen recognition, the interaction between PAMPs and PRRs on macrophages provides an intracellular signal that activates the phagocyte, causing it to transition from a dormant state of readiness and slow proliferation to a state of hyperactivity, proliferation, production/secretion of cytokines, and enhanced intracellular killing. PRRs on macrophages also respond to chemical distress signals from damaged or stressed cells. This allows macrophages to extend their responses beyond protection from infectious diseases to a broader role in the inflammatory response initiated from injuries or other diseases.



Phagocytic cells contain pattern recognition receptors (PRRs) capable of recognizing various pathogen-associated molecular patterns (PAMPs). These PRRs can be found on the plasma membrane or in internal phagosomes. When a PRR recognizes a PAMP, it sends a signal to the nucleus that activates genes involved in phagocytosis, cellular proliferation, production and secretion of antiviral interferons and proinflammatory cytokines, and enhanced intracellular killing.

Note:

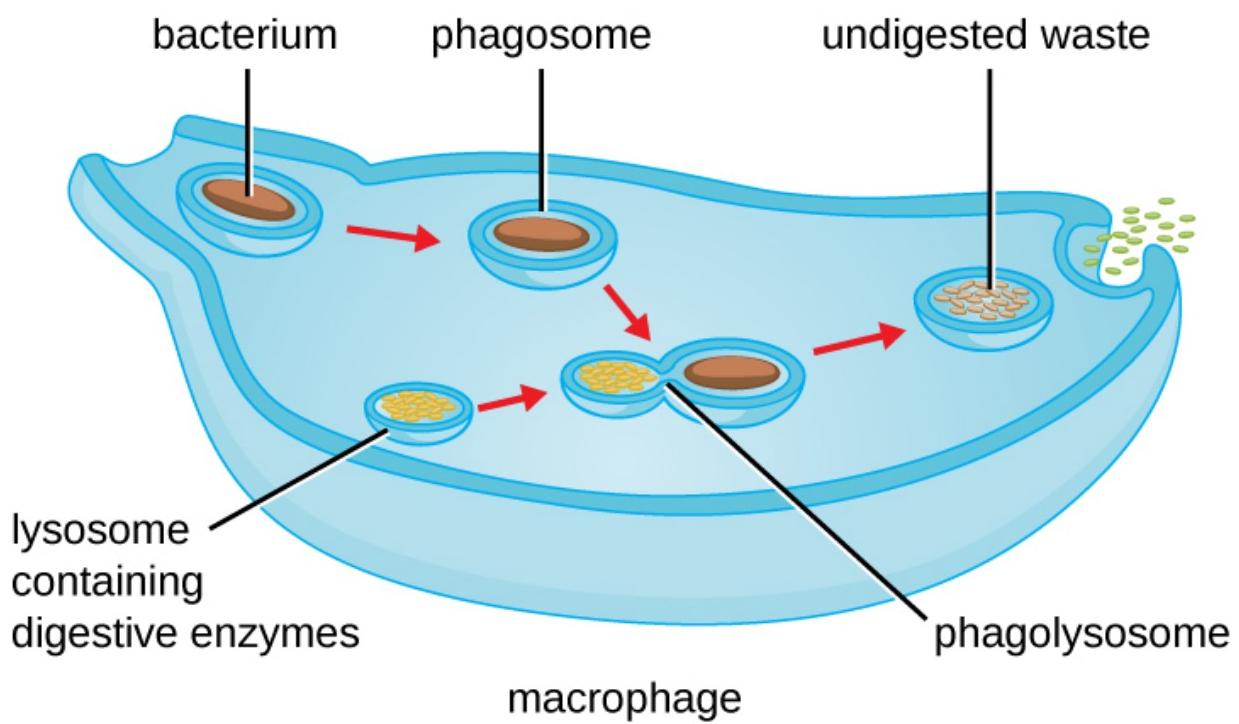
- Name four pathogen-associated molecular patterns (PAMPs).
- Describe the process of phagocyte activation.

Pathogen Degradation

Once pathogen recognition and attachment occurs, the pathogen is engulfed in a vesicle and brought into the internal compartment of the phagocyte in a process called **phagocytosis** ([\[link\]](#)). PRRs can aid in phagocytosis by first binding to the pathogen's surface, but phagocytes are also capable of engulfing nearby items even if they are not bound to specific receptors. To engulf the pathogen, the phagocyte forms a pseudopod that wraps around the pathogen and then pinches it off into a membrane vesicle called a **phagosome**. Acidification of the phagosome (pH decreases to the range of 4–5) provides an important early antibacterial mechanism. The phagosome containing the pathogen fuses with one or more lysosomes, forming a **phagolysosome**. Formation of the phagolysosome enhances the acidification, which is essential for activation of pH-dependent digestive lysosomal enzymes and production of hydrogen peroxide and toxic reactive oxygen species. Lysosomal enzymes such as lysozyme, phospholipase, and proteases digest the pathogen. Other enzymes are involved in a respiratory burst. During the respiratory burst, phagocytes will increase their uptake and consumption of oxygen, but not for energy production. The increased oxygen consumption is focused on the production of superoxide anion, hydrogen peroxide, hydroxyl radicals, and other reactive oxygen species that are antibacterial.

In addition to the reactive oxygen species produced by the respiratory burst, reactive nitrogen compounds with cytotoxic (cell-killing) potential can also form. For example, nitric oxide can react with superoxide to form peroxy nitrite, a highly reactive nitrogen compound with degrading capabilities similar to those of the reactive oxygen species. Some phagocytes even contain an internal storehouse of microbicidal defensin proteins (e.g., neutrophil granules). These destructive forces can be released into the area around the cell to degrade microbes externally. Neutrophils, especially, can be quite efficient at this secondary antimicrobial mechanism.

Once degradation is complete, leftover waste products are excreted from the cell in an exocytic vesicle. However, it is important to note that not all remains of the pathogen are excreted as waste. Macrophages and dendritic cells are also antigen-presenting cells involved in the specific adaptive immune response. These cells further process the remains of the degraded pathogen and present key antigens (specific pathogen proteins) on their cellular surface. This is an important step for stimulation of some adaptive immune responses, as will be discussed in more detail in the next chapter.



The stages of phagocytosis include the engulfment of a pathogen, the formation of a phagosome, the digestion of the pathogenic particle in the phagolysosome, and the expulsion of undigested materials from the cell.

Note:



Visit this [link](#) to view a phagocyte chasing and engulfing a pathogen.

Note:

- What is the difference between a phagosome and a lysosome?

Note:

When Phagocytosis Fails

Although phagocytosis successfully destroys many pathogens, some are able to survive and even exploit this defense mechanism to multiply in the body and cause widespread infection. Protozoans of the genus *Leishmania* are one example. These obligate intracellular parasites are flagellates transmitted to humans by the bite of a sand fly. Infections cause serious and sometimes disfiguring sores and ulcers in the skin and other tissues ([\[link\]](#)). Worldwide, an estimated 1.3 million people are newly infected with leishmaniasis annually.[\[footnote\]](#)

World Health Organization. “Leishmaniasis.” 2016.

<http://www.who.int/mediacentre/factsheets/fs375/en/>.

Salivary peptides from the sand fly activate host macrophages at the site of their bite. The classic or alternate pathway for complement activation ensues with C3b opsonization of the parasite. *Leishmania* cells are phagocytosed, lose their flagella, and multiply in a form known as an amastigote (Leishman-Donovan body) within the phagolysosome.

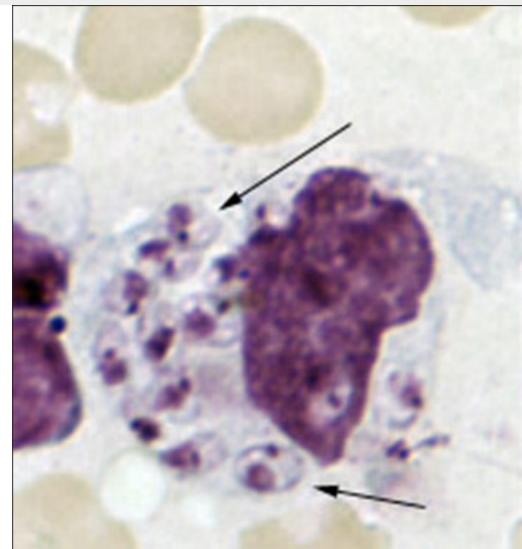
Although many other pathogens are destroyed in the phagolysosome, survival of the *Leishmania* amastigotes is maintained by the presence of

surface lipophosphoglycan and acid phosphatase. These substances inhibit the macrophage respiratory burst and lysosomal enzymes. The parasite then multiplies inside the cell and lyses the infected macrophage, releasing the amastigotes to infect other macrophages within the same host. Should another sand fly bite an infected person, it might ingest amastigotes and then transmit them to another individual through another bite.

There are several different forms of leishmaniasis. The most common is a localized cutaneous form of the illness caused by *L. tropica*, which typically resolves spontaneously over time but with some significant lymphocyte infiltration and permanent scarring. A mucocutaneous form of the disease, caused by *L. viannia brasiliensis*, produces lesions in the tissue of the nose and mouth and can be life threatening. A visceral form of the illness can be caused by several of the different *Leishmania* species. It affects various organ systems and causes abnormal enlargement of the liver and spleen. Irregular fevers, anemia, liver dysfunction, and weight loss are all signs and symptoms of visceral leishmaniasis. If left untreated, it is typically fatal.



(a)



(b)

(a) Cutaneous leishmaniasis is a disfiguring disease caused by the intracellular flagellate *Leishmania tropica*, transmitted by the bite of a sand fly. (b) This light micrograph of a sample taken from a skin lesion shows a large cell, which is a macrophage infected with *L. tropica* amastigotes (arrows). The amastigotes have lost their flagella but their nuclei are visible. Soon the amastigotes will lyse the

macrophage and be engulfed by other phagocytes, spreading the infection. (credit a: modification of work by Otis Historical Archives of “National Museum of Health & Medicine”; credit b: modification of work by Centers for Disease Control and Prevention)

Key Concepts and Summary

- Phagocytes are cells that recognize pathogens and destroy them through phagocytosis.
- Recognition often takes place by the use of phagocyte receptors that bind molecules commonly found on pathogens, known as **pathogen-associated molecular patterns (PAMPs)**.
- The receptors that bind PAMPs are called **pattern recognition receptors**, or **PRRs**. **Toll-like receptors (TLRs)** are one type of PRR found on phagocytes.
- **Extravasation** of white blood cells from the bloodstream into infected tissue occurs through the process of **transendothelial migration**.
- Phagocytes degrade pathogens through **phagocytosis**, which involves engulfing the pathogen, killing and digesting it within a **phagolysosome**, and then excreting undigested matter.

Short Answer

Exercise:

Problem:

Briefly summarize the events leading up to and including the process of transendothelial migration.

Inflammation and Fever

LEARNING OBJECTIVES

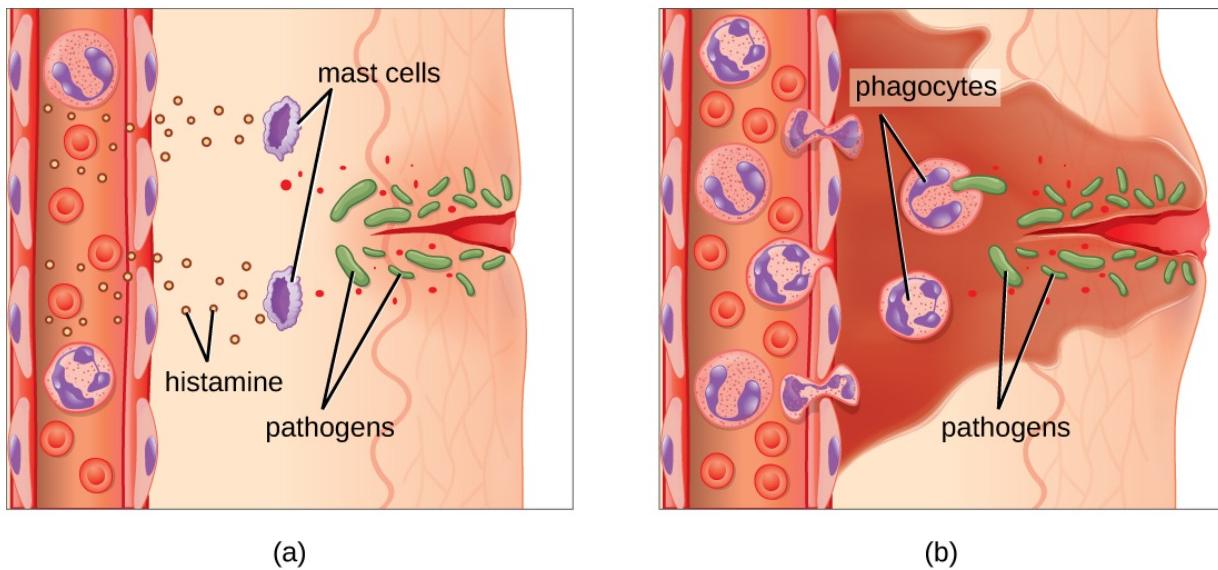
- Identify the signs of inflammation and fever and explain why they occur
- Explain the advantages and risks posed by inflammatory responses

The inflammatory response, or **inflammation**, is triggered by a cascade of chemical mediators and cellular responses that may occur when cells are damaged and stressed or when pathogens successfully breach the physical barriers of the innate immune system. Although inflammation is typically associated with negative consequences of injury or disease, it is a necessary process insofar as it allows for recruitment of the cellular defenses needed to eliminate pathogens, remove damaged and dead cells, and initiate repair mechanisms. Excessive inflammation, however, can result in local tissue damage and, in severe cases, may even become deadly.

Acute Inflammation

An early, if not immediate, response to tissue injury is acute inflammation. Immediately following an injury, vasoconstriction of blood vessels will occur to minimize blood loss. The amount of vasoconstriction is related to the amount of vascular injury, but it is usually brief. Vasoconstriction is followed by vasodilation and increased vascular permeability, as a direct result of the release of histamine from resident mast cells. Increased blood flow and vascular permeability can dilute toxins and bacterial products at the site of injury or infection. They also contribute to the five observable

signs associated with the inflammatory response: **erythema** (redness), **edema** (swelling), heat, pain, and altered function. Vasodilation and increased vascular permeability are also associated with an influx of phagocytes at the site of injury and/or infection. This can enhance the inflammatory response because phagocytes may release proinflammatory chemicals when they are activated by cellular distress signals released from damaged cells, by PAMPs, or by opsonins on the surface of pathogens. Activation of the complement system can further enhance the inflammatory response through the production of the anaphylatoxin C5a. [\[link\]](#) illustrates a typical case of acute inflammation at the site of a skin wound.



(a) Mast cells detect injury to nearby cells and release histamine, initiating an inflammatory response. (b) Histamine increases blood flow to the wound site, and increased vascular permeability allows fluid, proteins, phagocytes, and other immune cells to enter infected tissue. These events result in the swelling and reddening of the injured site, and the increased blood flow to the injured site causes it to feel warm. Inflammation is also associated with pain due to these events stimulating nerve pain receptors in the tissue. The interaction of phagocyte PRRs with cellular distress signals and PAMPs and opsonins on the surface of pathogens leads to the release of more proinflammatory chemicals, enhancing the inflammatory response.

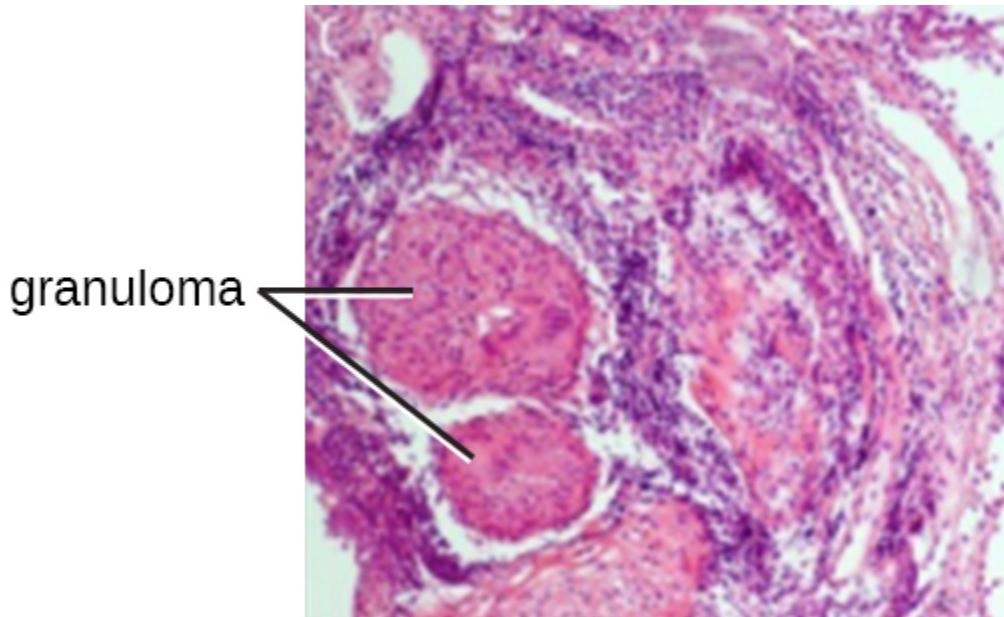
During the period of inflammation, the release of bradykinin causes capillaries to remain dilated, flooding tissues with fluids and leading to edema. Increasing numbers of neutrophils are recruited to the area to fight pathogens. As the fight rages on, pus forms from the accumulation of neutrophils, dead cells, tissue fluids, and lymph. Typically, after a few days, macrophages will help to clear out this pus. Eventually, tissue repair can begin in the wounded area.

Chronic Inflammation

When acute inflammation is unable to clear an infectious pathogen, chronic inflammation may occur. This often results in an ongoing (and sometimes futile) lower-level battle between the host organism and the pathogen. The wounded area may heal at a superficial level, but pathogens may still be present in deeper tissues, stimulating ongoing inflammation. Additionally, chronic inflammation may be involved in the progression of degenerative neurological diseases such as Alzheimer's and Parkinson's, heart disease, and metastatic cancer.

Chronic inflammation may lead to the formation of **granulomas**, pockets of infected tissue walled off and surrounded by WBCs. Macrophages and other phagocytes wage an unsuccessful battle to eliminate the pathogens and dead cellular materials within a granuloma. One example of a disease that produces chronic inflammation is tuberculosis, which results in the formation of granulomas in lung tissues. A tubercular granuloma is called a tubercle ([\[link\]](#)). Tuberculosis will be covered in more detail in [Bacterial Infections of the Respiratory Tract](#).

Chronic inflammation is not just associated with bacterial infections. Chronic inflammation can be an important cause of tissue damage from viral infections. The extensive scarring observed with hepatitis C infections and liver cirrhosis is the result of chronic inflammation.



A tubercle is a granuloma in the lung tissue of a patient with tuberculosis. In this micrograph, white blood cells (stained purple) have walled off a pocket of tissue infected with *Mycobacterium tuberculosis*. Granulomas also occur in many other forms of disease. (credit: modification of work by Piotrowski WJ, Górska P, Duda-Szymańska J, Kwiatkowska S)

Note:

- Name the five signs of inflammation.
- Is a granuloma an acute or chronic form of inflammation? Explain.

Note:

Chronic Edema

In addition to granulomas, chronic inflammation can also result in long-term edema. A condition known as lymphatic filariasis (also known as elephantiasis) provides an extreme example. Lymphatic filariasis is caused by microscopic nematodes (parasitic worms) whose larvae are transmitted between human hosts by mosquitoes. Adult worms live in the lymphatic vessels, where their presence stimulates infiltration by lymphocytes, plasma cells, eosinophils, and thrombocytes (a condition known as lymphangitis). Because of the chronic nature of the illness, granulomas, fibrosis, and blocking of the lymphatic system may eventually occur. Over time, these blockages may worsen with repeated infections over decades, leading to skin thickened with edema and fibrosis. Lymph (extracellular tissue fluid) may spill out of the lymphatic areas and back into tissues, causing extreme swelling ([\[link\]](#)). Secondary bacterial infections commonly follow. Because it is a disease caused by a parasite, eosinophilia (a dramatic rise in the number of eosinophils in the blood) is characteristic of acute infection. However, this increase in antiparasite granulocytes is not sufficient to clear the infection in many cases.

Lymphatic filariasis affects an estimated 120 million people worldwide, mostly concentrated in Africa and Asia.[\[footnote\]](#) Improved sanitation and mosquito control can reduce transmission rates.

Centers for Disease Control and Prevention. “Parasites—Lymphatic Filiariasis.” 2016.

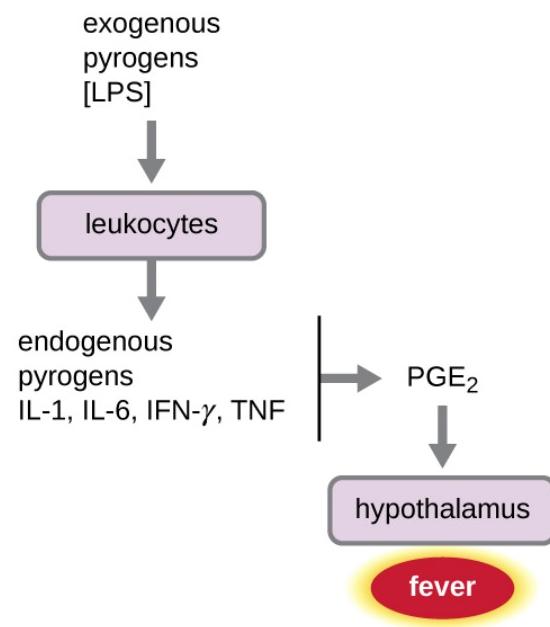
http://www.cdc.gov/parasites/lymphaticfilariasis/gen_info/faqs.html.



Elephantiasis (chronic edema) of the legs due to filariasis. (credit: modification of work by Centers for Disease Control and Prevention)

Fever

A **fever** is an inflammatory response that extends beyond the site of infection and affects the entire body, resulting in an overall increase in body temperature. Body temperature is normally regulated and maintained by the hypothalamus, an anatomical section of the brain that functions to maintain homeostasis in the body. However, certain bacterial or viral infections can result in the production of **pyrogens**, chemicals that effectively alter the “thermostat setting” of the hypothalamus to elevate body temperature and cause fever. Pyrogens may be exogenous or endogenous. For example, the endotoxin lipopolysaccharide (LPS), produced by gram-negative bacteria, is an exogenous pyrogen that may induce the leukocytes to release endogenous pyrogens such as interleukin-1 (IL-1), IL-6, interferon- γ (IFN- γ), and tumor necrosis factor (TNF). In a cascading effect, these molecules can then lead to the release of prostaglandin E2 (PGE_2) from other cells, resetting the hypothalamus to initiate fever ([\[link\]](#)).



The role of the hypothalamus in the inflammatory response. Macrophages recognize pathogens in an area and release cytokines that trigger inflammation. The cytokines also send a signal up the vagus nerve to the hypothalamus.

Like other forms of inflammation, a fever enhances the innate immune defenses by stimulating leukocytes to kill pathogens. The rise in body temperature also may inhibit the growth of many pathogens since human pathogens are mesophiles with optimum growth occurring around 35 °C (95 °F). In addition, some studies suggest that fever may also stimulate release of iron-sequestering compounds from the liver, thereby starving out microbes that rely on iron for growth.[\[footnote\]](#)

N. Parrow et al. “Sequestration and Scavenging of Iron in Infection.” *Infection and Immunity* 81 no. 10 (2013):3503–3514

During fever, the skin may appear pale due to vasoconstriction of the blood vessels in the skin, which is mediated by the hypothalamus to divert blood flow away from extremities, minimizing the loss of heat and raising the core temperature. The hypothalamus will also stimulate shivering of muscles, another effective mechanism of generating heat and raising the core temperature.

The **crisis phase** occurs when the fever breaks. The hypothalamus stimulates vasodilation, resulting in a return of blood flow to the skin and a subsequent release of heat from the body. The hypothalamus also stimulates sweating, which cools the skin as the sweat evaporates.

Although a low-level fever may help an individual overcome an illness, in some instances, this immune response can be too strong, causing tissue and organ damage and, in severe cases, even death. The inflammatory response to bacterial superantigens is one scenario in which a life-threatening fever may develop. Superantigens are bacterial or viral proteins that can cause an excessive activation of T cells from the specific adaptive immune defense, as well as an excessive release of cytokines that overstimulates the inflammatory response. For example, *Staphylococcus aureus* and *Streptococcus pyogenes* are capable of producing superantigens that cause toxic shock syndrome and scarlet fever, respectively. Both of these conditions can be associated with very high, life-threatening fevers in excess of 42 °C (108 °F).

Note:

- Explain the difference between exogenous and endogenous pyrogens.
- How does a fever inhibit pathogens?

Key Concepts and Summary

- **Inflammation** results from the collective response of chemical mediators and cellular defenses to an injury or infection.
- **Acute inflammation** is short lived and localized to the site of injury or infection. **Chronic inflammation** occurs when the inflammatory response is unsuccessful, and may result in the formation of **granulomas** (e.g., with tuberculosis) and scarring (e.g., with hepatitis C viral infections and liver cirrhosis).
- The five cardinal signs of inflammation are **erythema**, **edema**, heat, pain, and altered function. These largely result from innate responses that draw increased blood flow to the injured or infected tissue.
- **Fever** is a system-wide sign of inflammation that raises the body temperature and stimulates the immune response.
- Both inflammation and fever can be harmful if the inflammatory response is too severe.

Short Answer

Exercise:

Problem:

Differentiate exogenous and endogenous pyrogens, and provide an example of each.

Critical Thinking

Exercise:

Problem:

If a gram-negative bacterial infection reaches the bloodstream, large quantities of LPS can be released into the blood, resulting in a syndrome called septic shock. Death due to septic shock is a real danger. The overwhelming immune and inflammatory responses that occur with septic shock can cause a perilous drop in blood pressure; intravascular blood clotting; development of thrombi and emboli that block blood vessels, leading to tissue death; failure of multiple organs; and death of the patient. Identify and characterize two to three therapies that might be useful in stopping the dangerous events and outcomes of septic shock once it has begun, given what you have learned about inflammation and innate immunity in this chapter.

Exercise:**Problem:**

In Lubeck, Germany, in 1930, a group of 251 infants was accidentally administered a tainted vaccine for tuberculosis that contained live *Mycobacterium tuberculosis*. This vaccine was administered orally, directly exposing the infants to the deadly bacterium. Many of these infants contracted tuberculosis, and some died. However, 44 of the infants never contracted tuberculosis. Based on your knowledge of the innate immune system, what innate defenses might have inhibited *M. tuberculosis* enough to prevent these infants from contracting the disease?

Adaptive Immunity - Introduction

class="introduction"

Polio was once a common disease with potentially serious consequences, including paralysis. Vaccination has all but eliminated the disease from most countries around the world. An iron-lung ward, such as the one shown in this 1953 photograph, housed patients paralyzed from polio and unable to breathe for themselves.



People living in developed nations and born in the 1960s or later may have difficulty understanding the once heavy burden of devastating infectious diseases. For example, smallpox, a deadly viral disease, once destroyed entire civilizations but has since been eradicated. Thanks to the vaccination efforts by multiple groups, including the World Health Organization, Rotary International, and the United Nations Children's Fund (UNICEF), smallpox has not been diagnosed in a patient since 1977. Polio is another excellent example. This crippling viral disease paralyzed patients, who were often kept alive in “iron lung wards” as recently as the 1950s ([\[link\]](#)). Today, vaccination against polio has nearly eradicated the disease. Vaccines have also reduced the prevalence of once-common infectious diseases such as chickenpox, German measles, measles, mumps, and whooping cough. The success of these and other vaccines is due to the very specific and adaptive host defenses that are the focus of this chapter.

[Innate Nonspecific Host Defenses](#) described innate immunity against microbial pathogens. Higher animals, such as humans, also possess an **adaptive immune defense**, which is highly specific for individual microbial pathogens. This specific adaptive immunity is acquired through active infection or vaccination and serves as an important defense against pathogens that evade the defenses of innate immunity.

Overview of Adaptive Immunity

LEARNING OBJECTIVES

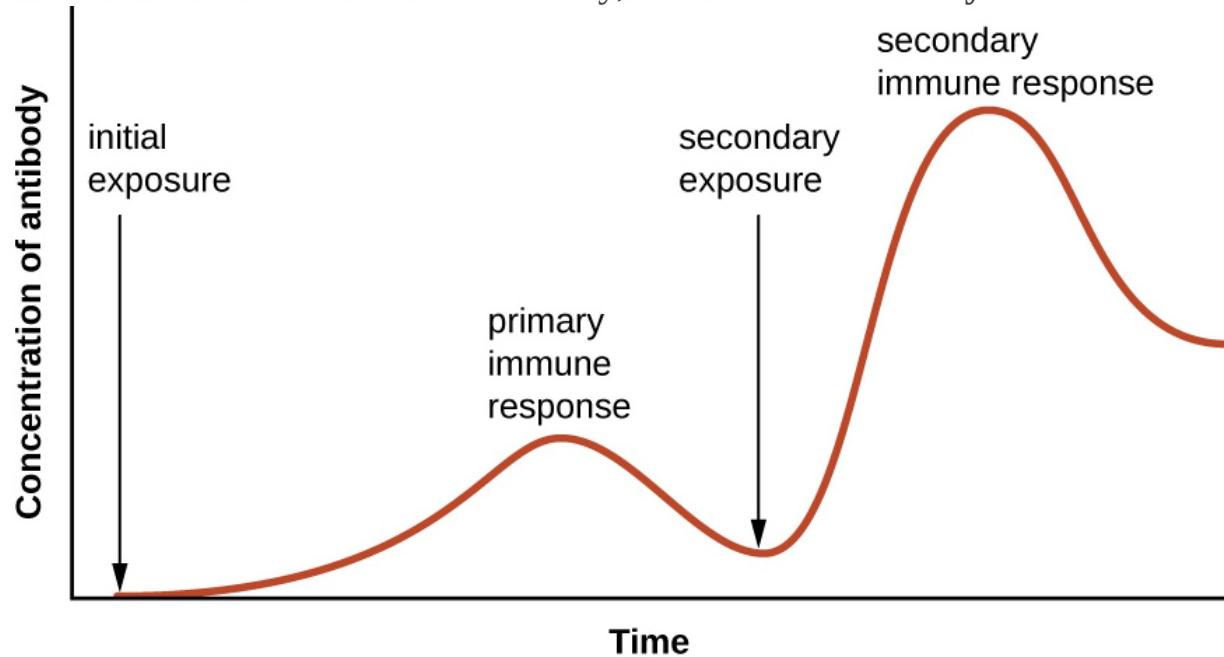
- Define memory, primary response, secondary response, and specificity
- Distinguish between humoral and cellular immunity
- Differentiate between antigens, epitopes, and haptens
- Describe the structure and function of antibodies and distinguish between the different classes of antibodies
- Describe the interactions between antibodies and antigens

Adaptive immunity is defined by two important characteristics: **specificity** and **memory**. Specificity refers to the adaptive immune system's ability to target specific pathogens, and memory refers to its ability to quickly respond to pathogens to which it has previously been exposed. For example, when an individual recovers from chickenpox, the body develops a *memory* of the infection that will *specifically* protect it from the causative agent, the varicella-zoster virus, if it is exposed to the virus again later.

Specificity and memory are achieved by essentially programming certain cells involved in the immune response to respond rapidly to subsequent exposures of the pathogen. This programming occurs as a result of the first exposure to a pathogen or vaccine, which triggers a **primary response**. Subsequent exposures result in a **secondary response** that is faster and stronger as a result of the body's memory of the first exposure ([\[link\]](#)). This secondary response, however, is specific to the pathogen in question. For example, exposure to one virus (e.g., varicella-zoster virus) will not provide protection against other viral diseases (e.g., measles, mumps, or polio).

Adaptive specific immunity involves the actions of two distinct cell types: **B lymphocytes (B cells)** and **T lymphocytes (T cells)**. Although B cells and T cells arise from a common hematopoietic stem cell differentiation pathway (see [[link](#)]), their sites of maturation and their roles in adaptive immunity are very different.

B cells mature in the bone marrow and are responsible for the production of glycoproteins called **antibodies**, or **immunoglobulins**. Antibodies are involved in the body's defense against pathogens and toxins in the extracellular environment. Mechanisms of adaptive specific immunity that involve B cells and antibody production are referred to as **humoral immunity**. The maturation of T cells occurs in the thymus. T cells function as the central orchestrator of both innate and adaptive immune responses. They are also responsible for destruction of cells infected with intracellular pathogens. The targeting and destruction of intracellular pathogens by T cells is called cell-mediated immunity, or **cellular immunity**.



This graph illustrates the primary and secondary immune responses related to antibody production after an initial and secondary exposure to an antigen. Notice that the secondary response is faster and provides a much higher concentration of antibody.

Note:

- List the two defining characteristics of adaptive immunity.
- Explain the difference between a primary and secondary immune response.
- How do humoral and cellular immunity differ?

Antigens

Activation of the adaptive immune defenses is triggered by pathogen-specific molecular structures called **antigens**. Antigens are similar to the pathogen-associated molecular patterns (PAMPs) discussed in [Pathogen Recognition and Phagocytosis](#); however, whereas PAMPs are molecular structures found on numerous pathogens, antigens are unique to a specific pathogen. The antigens that stimulate adaptive immunity to chickenpox, for example, are unique to the varicella-zoster virus but significantly different from the antigens associated with other viral pathogens.

The term *antigen* was initially used to describe molecules that stimulate the production of antibodies; in fact, the term comes from a combination of the words antibody and generator, and a molecule that stimulates antibody production is said to be **antigenic**. However, the role of antigens is not limited to humoral immunity and the production of antibodies; antigens also play an essential role in stimulating cellular immunity, and for this reason antigens are sometimes more accurately referred to as **immunogens**. In this text, however, we will typically refer to them as antigens.

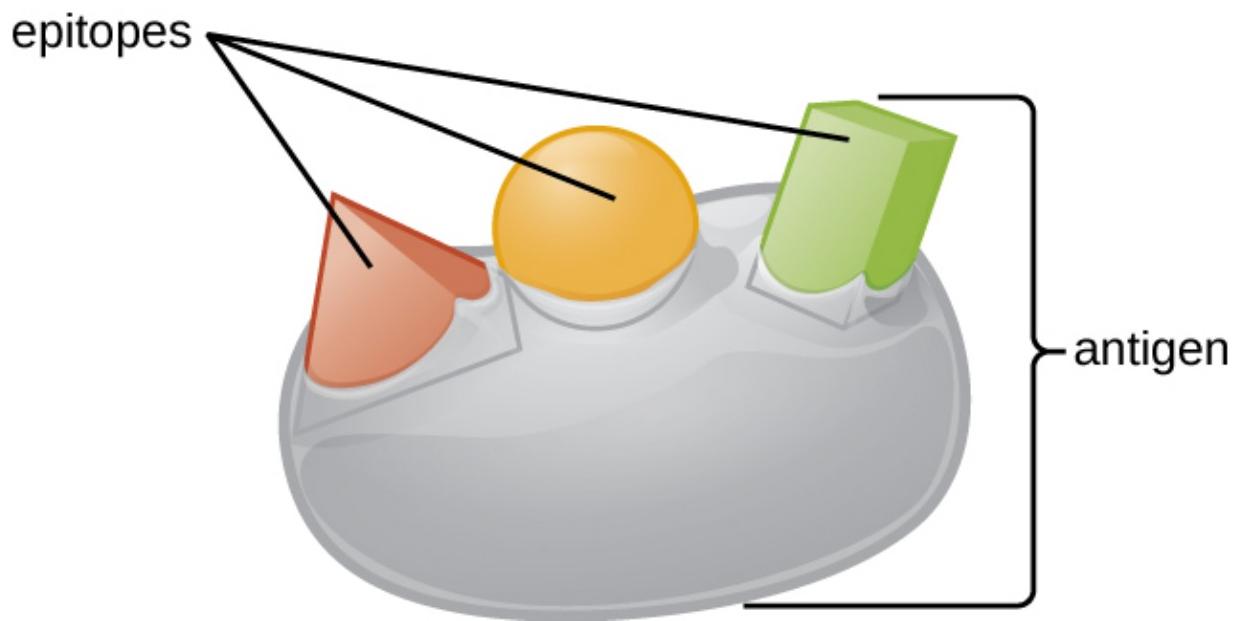
Pathogens possess a variety of structures that may contain antigens. For example, antigens from bacterial cells may be associated with their capsules, cell walls, fimbriae, flagella, or pili. Bacterial antigens may also be associated with extracellular toxins and enzymes that they secrete. Viruses possess a variety of antigens associated with their capsids, envelopes, and the spike structures they use for attachment to cells.

Antigens may belong to any number of molecular classes, including carbohydrates, lipids, nucleic acids, proteins, and combinations of these molecules. Antigens of different classes vary in their ability to stimulate adaptive immune defenses as well as in the type of response they stimulate (humoral or cellular). The structural complexity of an antigenic molecule is an important factor in its antigenic potential. In general, more complex molecules are more effective as antigens. For example, the three-dimensional complex structure of proteins make them the most effective and potent antigens, capable of stimulating both humoral and cellular immunity. In comparison, carbohydrates are less complex in structure and therefore less effective as antigens; they can only stimulate humoral immune defenses. Lipids and nucleic acids are the least antigenic molecules, and in some cases may only become antigenic when combined with proteins or carbohydrates to form glycolipids, lipoproteins, or nucleoproteins.

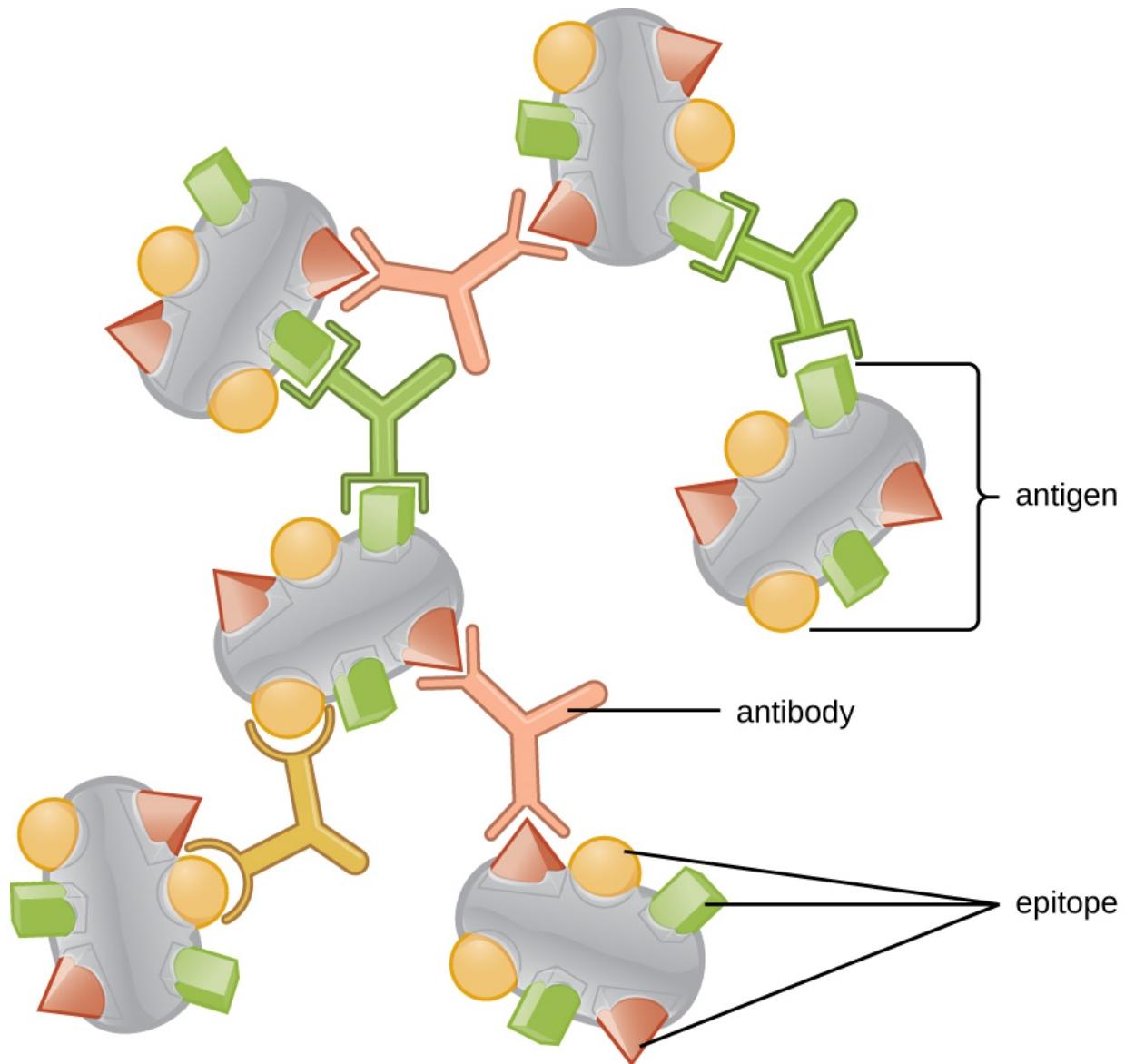
One reason the three-dimensional complexity of antigens is so important is that antibodies and T cells do not recognize and interact with an entire antigen but with smaller exposed regions on the surface of antigens called **epitopes**. A single antigen may possess several different epitopes ([\[link\]](#)), and different antibodies may bind to different epitopes on the same antigen ([\[link\]](#)). For example, the bacterial flagellum is a large, complex protein structure that can possess hundreds or even thousands of epitopes with unique three-dimensional structures. Moreover, flagella from different bacterial species (or even strains of the same species) contain unique epitopes that can only be bound by specific antibodies.

An antigen's size is another important factor in its antigenic potential. Whereas large antigenic structures like flagella possess multiple epitopes, some molecules are too small to be antigenic by themselves. Such molecules, called **hapto**s, are essentially free epitopes that are not part of the complex three-dimensional structure of a larger antigen. For a hapten to become antigenic, it must first attach to a larger carrier molecule (usually a protein) to produce a conjugate antigen. The haptene-specific antibodies produced in response to the conjugate antigen are then able to interact with unconjugated free haptene molecules. Haptene are not known to be associated with any specific pathogens, but they are responsible for some

allergic responses. For example, the hapten urushiol, a molecule found in the oil of plants that cause poison ivy, causes an immune response that can result in a severe rash (called contact dermatitis). Similarly, the hapten penicillin can cause allergic reactions to drugs in the penicillin class.



An antigen is a macromolecule that reacts with components of the immune system. A given antigen may contain several motifs that are recognized by immune cells.



A typical protein antigen has multiple epitopes, shown by the ability of three different antibodies to bind to different epitopes of the same antigen.

Note:

- What is the difference between an antigen and an epitope?

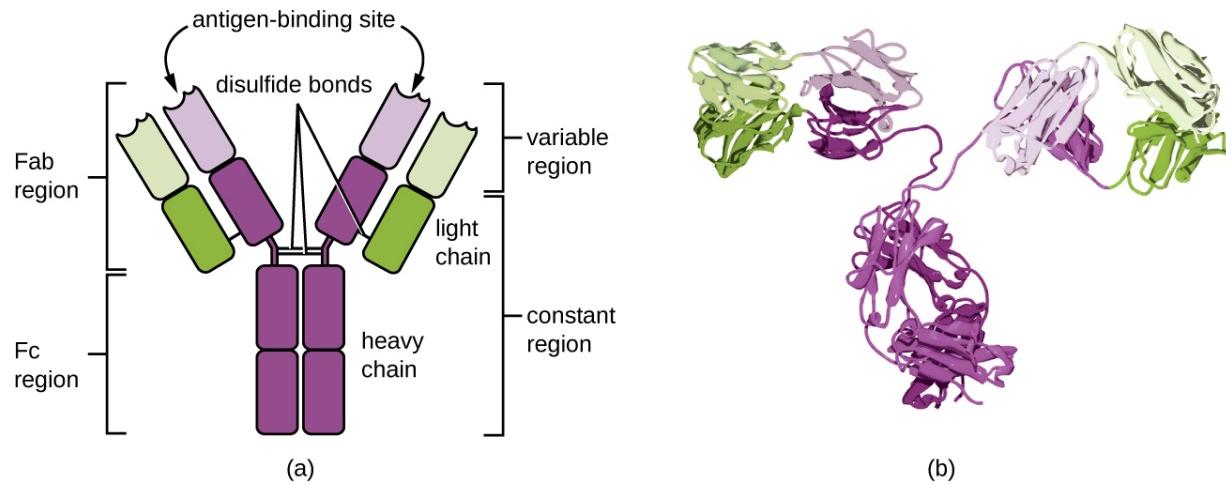
- What factors affect an antigen's antigenic potential?
- Why are haptens typically not antigenic, and how do they become antigenic?

Antibodies

Antibodies (also called immunoglobulins) are glycoproteins that are present in both the blood and tissue fluids. The basic structure of an antibody monomer consists of four protein chains held together by disulfide bonds ([\[link\]](#)). A disulfide bond is a covalent bond between the sulphydryl *R* groups found on two cysteine amino acids. The two largest chains are identical to each other and are called the **heavy chains**. The two smaller chains are also identical to each other and are called the **light chains**. Joined together, the heavy and light chains form a basic Y-shaped structure.

The two ‘arms’ of the Y-shaped antibody molecule are known as the **Fab region**, for “fragment of antigen binding.” The far end of the Fab region is the variable region, which serves as the site of antigen binding. The amino acid sequence in the variable region dictates the three-dimensional structure, and thus the specific three-dimensional epitope to which the Fab region is capable of binding. Although the epitope specificity of the Fab regions is identical for each arm of a single antibody molecule, this region displays a high degree of variability between antibodies with different epitope specificities. Binding to the Fab region is necessary for neutralization of pathogens, agglutination or aggregation of pathogens, and antibody-dependent cell-mediated cytotoxicity.

The constant region of the antibody molecule includes the trunk of the Y and lower portion of each arm of the Y. The trunk of the Y is also called the **Fc region**, for “fragment of crystallization,” and is the site of complement factor binding and binding to phagocytic cells during antibody-mediated opsonization.



(a) The typical four-chain structure of a generic antibody monomer. (b) The corresponding three-dimensional structure of the antibody IgG.
 (credit b: modification of work by Tim Vickers)

Note:

- Describe the different functions of the Fab region and the Fc region.

Antibody Classes

The constant region of an antibody molecule determines its class, or isotype. The five classes of antibodies are IgG, IgM, IgA, IgD, and IgE. Each class possesses unique heavy chains designated by Greek letters γ , μ , α , δ , and ϵ , respectively. Antibody classes also exhibit important differences in abundance in serum, arrangement, body sites of action, functional roles, and size ([\[link\]](#)).

IgG is a monomer that is by far the most abundant antibody in human blood, accounting for about 80% of total serum antibody. IgG penetrates efficiently into tissue spaces, and is the only antibody class with the ability

to cross the placental barrier, providing passive immunity to the developing fetus during pregnancy. IgG is also the most versatile antibody class in terms of its role in the body's defense against pathogens and occurs in large numbers during the secondary antibody response.

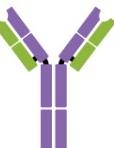
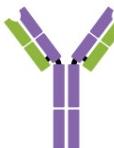
IgM is initially produced in a monomeric membrane-bound form that serves as an antigen-binding receptor on B cells. The secreted form of IgM assembles into a pentamer with five monomers of IgM bound together by a protein structure called the J chain. Although the location of the J chain relative to the Fc regions of the five monomers prevents IgM from performing some of the functions of IgG, the ten available Fab sites associated with a pentameric IgM make it an important antibody in the body's arsenal of defenses. IgM is the first antibody produced and secreted by B cells during the primary immune responses, making pathogen-specific IgM a valuable diagnostic marker during active or recent infections.

IgA accounts for about 13% of total serum antibody, and secretory IgA is the most common and abundant antibody class found in the mucus secretions that protect the mucous membranes. IgA can also be found in other secretions such as breast milk, tears, and saliva. Secretory IgA is assembled into a dimeric form with two monomers joined by a protein structure called the secretory component. One of the important functions of secretory IgA is to trap pathogens in mucus so that they can later be eliminated from the body.

Similar to IgM, **IgD** is a membrane-bound monomer found on the surface of B cells, where it serves as an antigen-binding receptor. However, IgD is not secreted by B cells, and only trace amounts are detected in serum. These trace amounts most likely come from the degradation of old B cells and the release of IgD molecules from their cytoplasmic membranes.

IgE is the least abundant antibody class in serum. Like IgG, it is secreted as a monomer, but its role in adaptive immunity is restricted to anti-parasitic defenses. The Fc region of IgE binds to basophils and mast cells. The Fab region of the bound IgE then interacts with specific antigen epitopes, causing the cells to release potent pro-inflammatory mediators. The inflammatory reaction resulting from the activation of mast cells and

basophils aids in the defense against parasites, but this reaction is also central to allergic reactions (see [Diseases of the Immune System](#)).

The Five Immunoglobulin (Ig) Classes					
Properties	IgG monomer	IgM pentamer	Secretory IgA dimer	IgD monomer	IgE monomer
Structure					
Heavy chains	γ	μ	α	δ	ϵ
Number of antigen-binding sites	2	10	4	2	2
Molecular weight (Daltons)	150,000	900,000	385,000	180,000	200,000
Percentage of total antibody in serum	80%	6%	13% (monomer)	<1%	<1%
Crosses placenta	yes	no	no	no	no
Fixes complement	yes	yes	no	no	no
Fc binds to	phagocytes				mast cells and basophils
Function	Neutralization, agglutination, complement activation, opsonization, and antibody-dependent cell-mediated cytotoxicity.	Neutralization, agglutination, and complement activation. The monomer form serves as the B-cell receptor.	Neutralization and trapping of pathogens in mucus.	B-cell receptor.	Activation of basophils and mast cells against parasites and allergens.

Note:

- What part of an antibody molecule determines its class?
- What class of antibody is involved in protection against parasites?
- Describe the difference in structure between IgM and IgG.

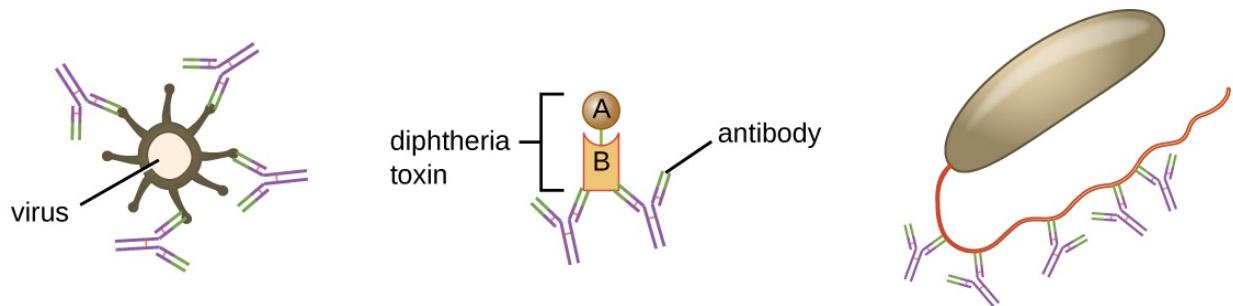
Antigen-Antibody Interactions

Different classes of antibody play important roles in the body's defense against pathogens. These functions include neutralization of pathogens, opsonization for phagocytosis, agglutination, complement activation, and antibody-dependent cell-mediated cytotoxicity. For most of these functions, antibodies also provide an important link between adaptive specific immunity and innate nonspecific immunity.

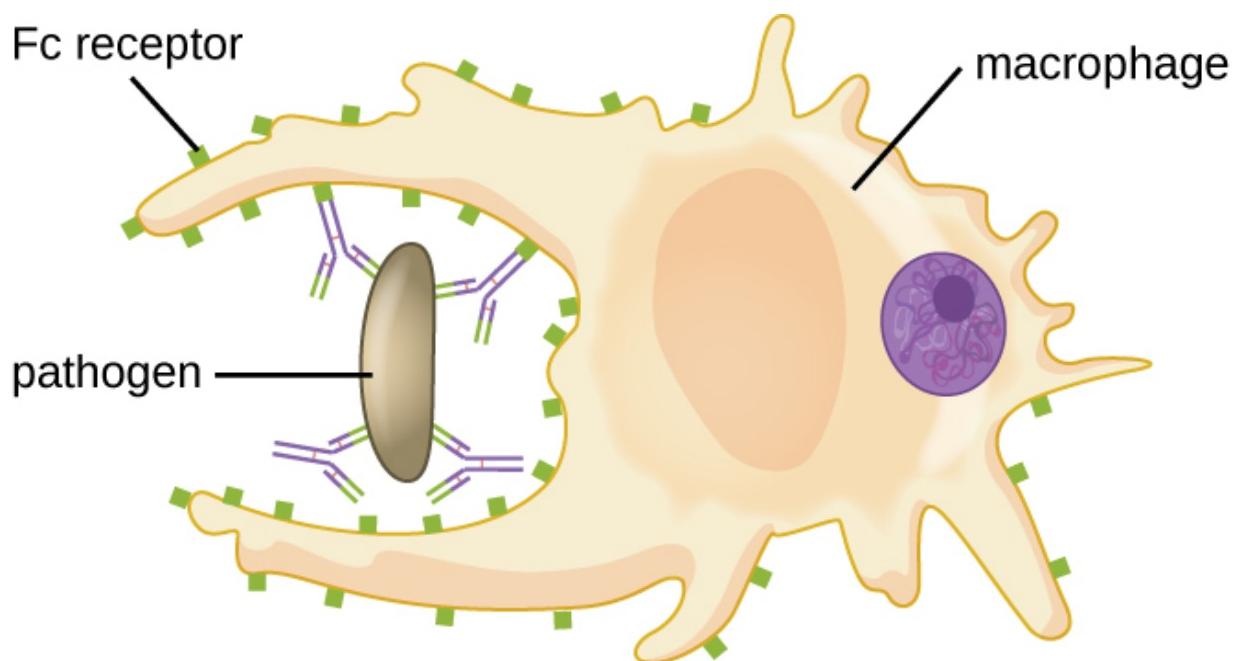
Neutralization involves the binding of certain antibodies (IgG, IgM, or IgA) to epitopes on the surface of pathogens or toxins, preventing their attachment to cells. For example, Secretory IgA can bind to specific pathogens and block initial attachment to intestinal mucosal cells. Similarly, specific antibodies can bind to certain toxins, blocking them from attaching to target cells and thus neutralizing their toxic effects. Viruses can be neutralized and prevented from infecting a cell by the same mechanism ([\[link\]](#)).

As described in [Chemical Defenses](#), opsonization is the coating of a pathogen with molecules, such as complement factors, C-reactive protein, and serum amyloid A, to assist in phagocyte binding to facilitate phagocytosis. IgG antibodies also serve as excellent opsonins, binding their Fab sites to specific epitopes on the surface of pathogens. Phagocytic cells such as macrophages, dendritic cells, and neutrophils have receptors on their surfaces that recognize and bind to the Fc portion of the IgG molecules; thus, IgG helps such phagocytes attach to and engulf the pathogens they have bound ([\[link\]](#)).

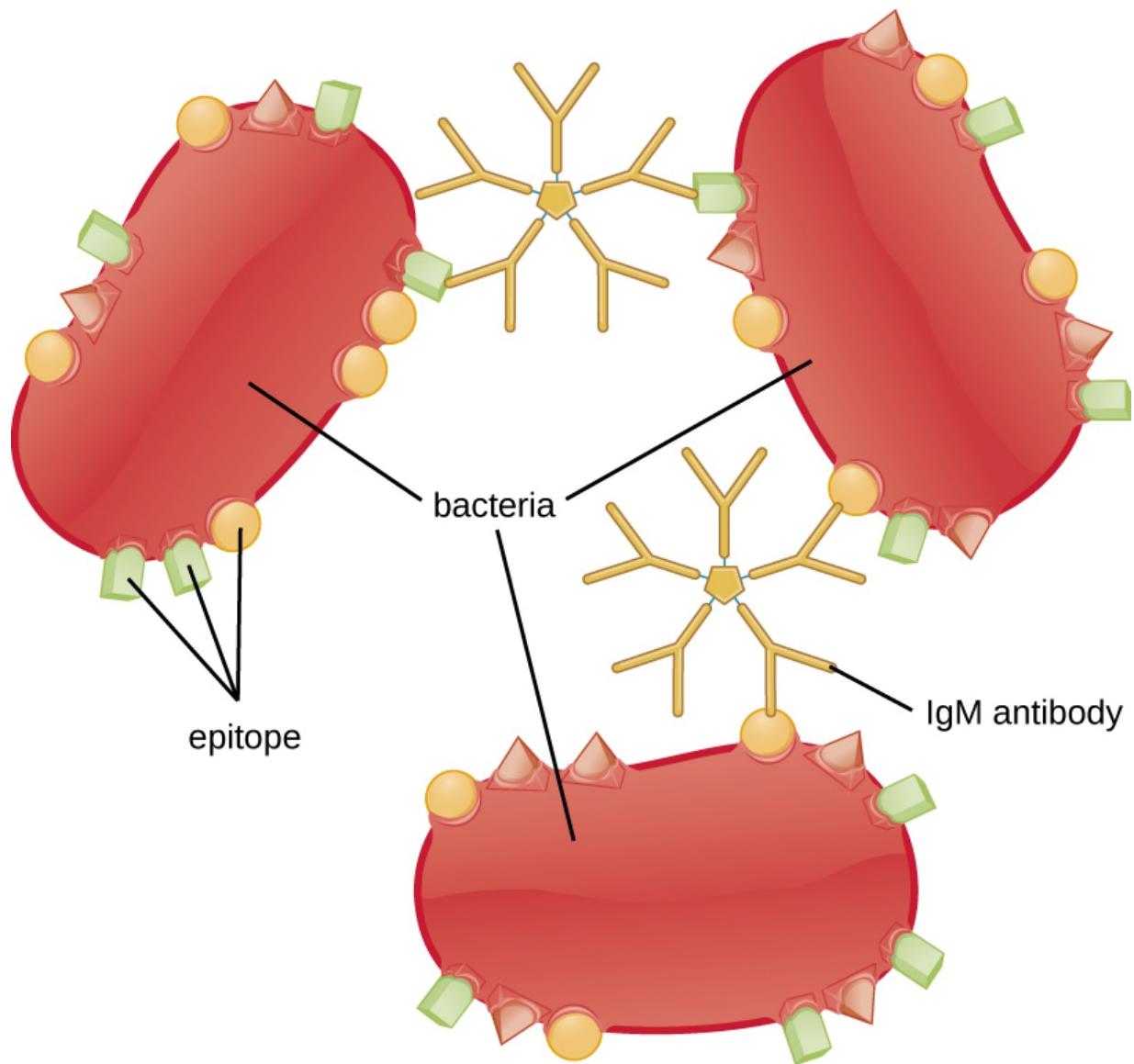
Agglutination or aggregation involves the cross-linking of pathogens by antibodies to create large aggregates ([\[link\]](#)). IgG has two Fab antigen-binding sites, which can bind to two separate pathogen cells, clumping them together. When multiple IgG antibodies are involved, large aggregates can develop; these aggregates are easier for the kidneys and spleen to filter from the blood and easier for phagocytes to ingest for destruction. The pentameric structure of IgM provides ten Fab binding sites per molecule, making it the most efficient antibody for agglutination.



Neutralization involves the binding of specific antibodies to antigens found on bacteria, viruses, and toxins, preventing them from attaching to target cells.



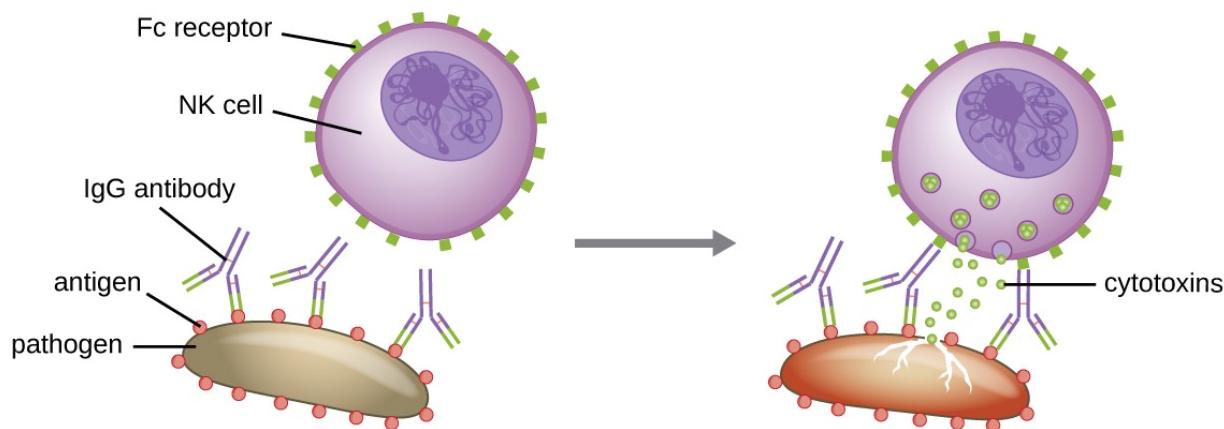
Antibodies serve as opsonins and inhibit infection by tagging pathogens for destruction by macrophages, dendritic cells, and neutrophils. These phagocytic cells use Fc receptors to bind to IgG-opsonized pathogens and initiate the first step of attachment before phagocytosis.



Antibodies, especially IgM antibodies, agglutinate bacteria by binding to epitopes on two or more bacteria simultaneously. When multiple pathogens and antibodies are present, aggregates form when the binding sites of antibodies bind with separate pathogens.

Another important function of antibodies is activation of the complement cascade. As discussed in the previous chapter, the complement system is an important component of the innate defenses, promoting the inflammatory response, recruiting phagocytes to site of infection, enhancing phagocytosis by opsonization, and killing gram-negative bacterial pathogens with the membrane attack complex (MAC). Complement activation can occur through three different pathways (see [\[link\]](#)), but the most efficient is the classical pathway, which requires the initial binding of IgG or IgM antibodies to the surface of a pathogen cell, allowing for recruitment and activation of the C1 complex.

Yet another important function of antibodies is **antibody-dependent cell-mediated cytotoxicity (ADCC)**, which enhances killing of pathogens that are too large to be phagocytosed. This process is best characterized for natural killer cells (NK cells), as shown in [\[link\]](#), but it can also involve macrophages and eosinophils. ADCC occurs when the Fab region of an IgG antibody binds to a large pathogen; Fc receptors on effector cells (e.g., NK cells) then bind to the Fc region of the antibody, bringing them into close proximity with the target pathogen. The effector cell then secretes powerful cytotoxins (e.g., perforin and granzymes) that kill the pathogen.



In this example of ADCC, antibodies bind to a large pathogenic cell that is too big for phagocytosis and then bind to Fc receptors on the membrane of a natural killer cell. This interaction brings the NK cell

into close proximity, where it can kill the pathogen through release of lethal extracellular cytotoxins.

Note:

- Where is IgA normally found?
- Which class of antibody crosses the placenta, providing protection to the fetus?
- Which class of antibody is passed from mother to child via colostrum?
- Compare the mechanisms of opsonization and antibody-dependent cell-mediated cytotoxicity.

Key Concepts and Summary

- **Adaptive immunity** is an acquired defense against foreign pathogens that is characterized by **specificity** and **memory**. The first exposure to an antigen stimulates a **primary response**, and subsequent exposures stimulate a faster and strong **secondary response**.
- Adaptive immunity is a dual system involving **humoral immunity** (antibodies produced by B cells) and **cellular immunity** (T cells directed against intracellular pathogens).
- **Antigens**, also called **immunogens**, are molecules that activate adaptive immunity. A single antigen possesses smaller **epitopes**, each capable of inducing a specific adaptive immune response.
- An antigen's ability to stimulate an immune response depends on several factors, including its molecular class, molecular complexity, and size.
- **Antibodies (immunoglobulins)** are Y-shaped glycoproteins with two Fab sites for binding antigens and an Fc portion involved in complement activation and opsonization.

- The five classes of antibody are **IgM**, **IgG**, **IgA**, **IgE**, and **IgD**, each differing in size, arrangement, location within the body, and function. The five primary functions of antibodies are neutralization, opsonization, agglutination, complement activation, and antibody-dependent cell-mediated cytotoxicity (ADCC).

Major Histocompatibility Complexes and Antigen-Presenting Cells

LEARNING OBJECTIVES

- Identify cells that express MHC I and/or MHC II molecules and describe the structures and cellular location of MHC I and MHC II molecules
- Identify the cells that are antigen-presenting cells
- Describe the process of antigen processing and presentation with MHC I and MHC II

As discussed in [Cellular Defenses](#), major histocompatibility complex (MHC) molecules are expressed on the surface of healthy cells, identifying them as normal and “self” to natural killer (NK) cells. MHC molecules also play an important role in the presentation of foreign antigens, which is a critical step in the activation of T cells and thus an important mechanism of the adaptive immune system.

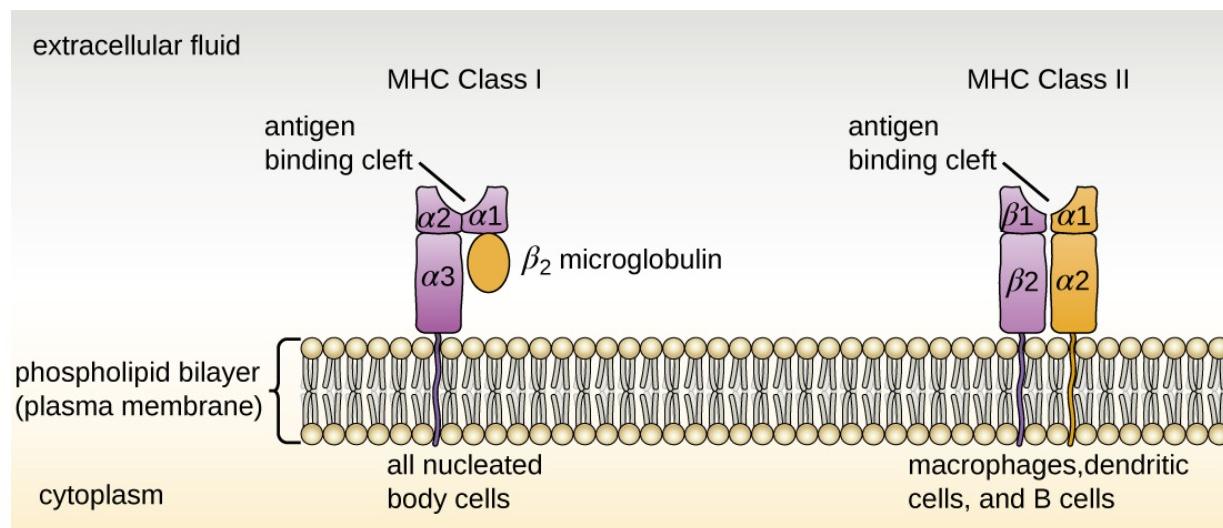
Major Histocompatibility Complex Molecules

The **major histocompatibility complex (MHC)** is a collection of genes coding for MHC molecules found on the surface of all nucleated cells of the body. In humans, the MHC genes are also referred to as human leukocyte antigen (HLA) genes. Mature red blood cells, which lack a nucleus, are the only cells that do not express MHC molecules on their surface.

There are two classes of MHC molecules involved in adaptive immunity, MHC I and MHC II ([\[link\]](#)). **MHC I** molecules are found on all nucleated cells; they present normal self-antigens as well as abnormal or nonself

pathogens to the effector T cells involved in cellular immunity. In contrast, **MHC II** molecules are only found on macrophages, dendritic cells, and B cells; they present abnormal or nonself pathogen antigens for the initial activation of T cells.

Both types of MHC molecules are transmembrane glycoproteins that assemble as dimers in the cytoplasmic membrane of cells, but their structures are quite different. MHC I molecules are composed of a longer α protein chain coupled with a smaller β_2 microglobulin protein, and only the α chain spans the cytoplasmic membrane. The α chain of the MHC I molecule folds into three separate domains: α_1 , α_2 and α_3 . MHC II molecules are composed of two protein chains (an α and a β chain) that are approximately similar in length. Both chains of the MHC II molecule possess portions that span the plasma membrane, and each chain folds into two separate domains: α_1 and α_2 , and β_1 , and β_2 . In order to present abnormal or non-self-antigens to T cells, MHC molecules have a cleft that serves as the antigen-binding site near the “top” (or outermost) portion of the MHC-I or MHC-II dimer. For MHC I, the antigen-binding cleft is formed by the α_1 and α_2 domains, whereas for MHC II, the cleft is formed by the α_1 and β_1 domains ([\[link\]](#)).



MHC I are found on all nucleated body cells, and MHC II are found on macrophages, dendritic cells, and B cells (along with MHC I). The

antigen-binding cleft of MHC I is formed by domains α_1 and α_2 . The antigen-binding cleft of MHC II is formed by domains α_1 and β_1 .

Note:

- Compare the structures of the MHC I and MHC II molecules.

Antigen-Presenting Cells (APCs)

All nucleated cells in the body have mechanisms for processing and presenting antigens in association with MHC molecules. This signals the immune system, indicating whether the cell is normal and healthy or infected with an intracellular pathogen. However, only macrophages, dendritic cells, and B cells have the ability to present antigens specifically for the purpose of activating T cells; for this reason, these types of cells are sometimes referred to as **antigen-presenting cells (APCs)**.

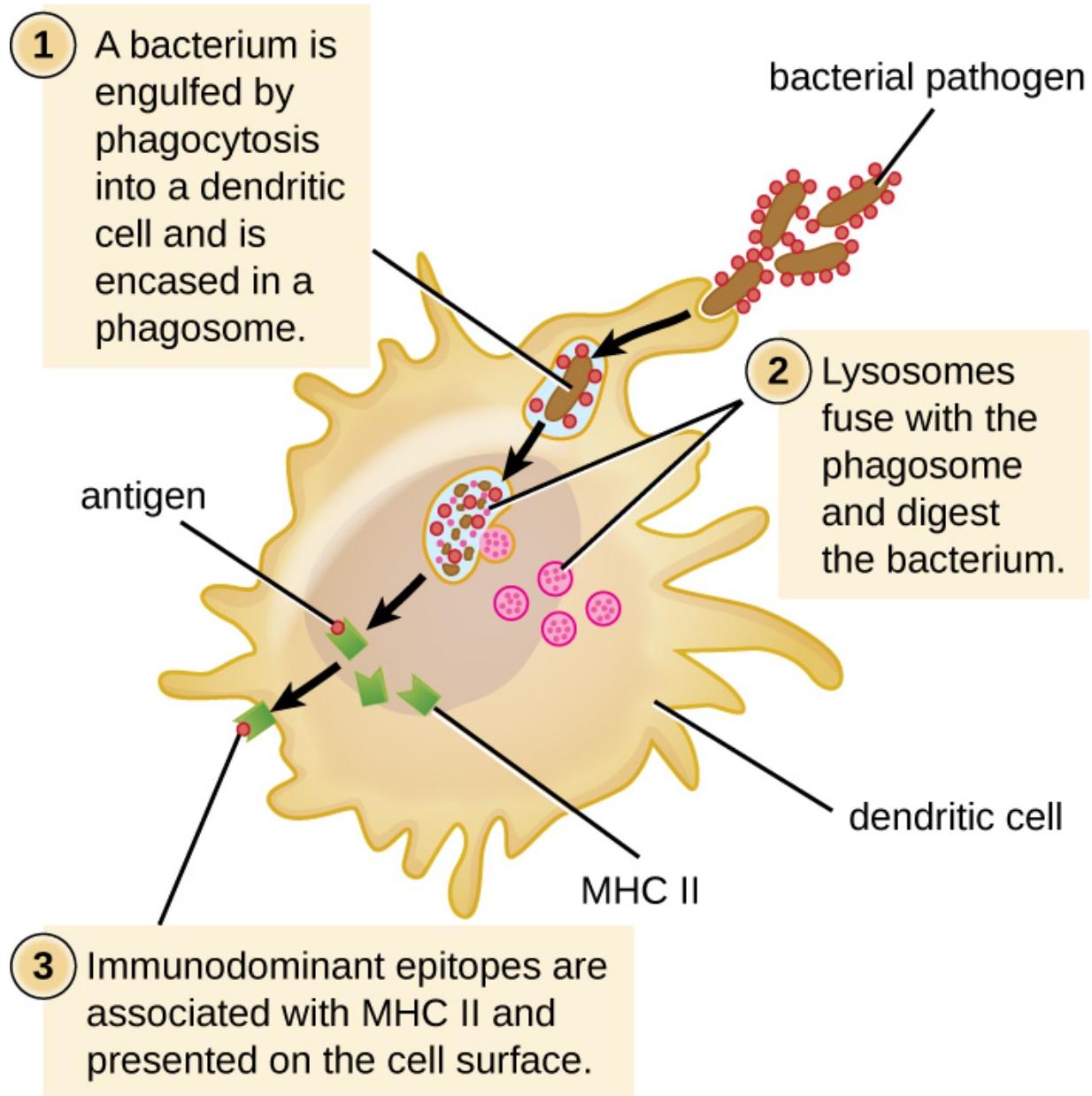
While all APCs play a similar role in adaptive immunity, there are some important differences to consider. Macrophages and dendritic cells are phagocytes that ingest and kill pathogens that penetrate the first-line barriers (i.e., skin and mucous membranes). B cells, on the other hand, do not function as phagocytes but play a primary role in the production and secretion of antibodies. In addition, whereas macrophages and dendritic cells recognize pathogens through nonspecific receptor interactions (e.g., PAMPs, toll-like receptors, and receptors for opsonizing complement or antibody), B cells interact with foreign pathogens or their free antigens using antigen-specific immunoglobulin as receptors (monomeric IgD and IgM). When the immunoglobulin receptors bind to an antigen, the B cell internalizes the antigen by endocytosis before processing and presenting the antigen to T cells.

Antigen Presentation with MHC II Molecules

MHC II molecules are only found on the surface of APCs. Macrophages and dendritic cells use similar mechanisms for processing and presentation of antigens and their epitopes in association with MHC II; B cells use somewhat different mechanisms that will be described further in [B Lymphocytes and Humoral Immunity](#). For now, we will focus on the steps of the process as they pertain to dendritic cells.

After a dendritic cell recognizes and attaches to a pathogen cell, the pathogen is internalized by phagocytosis and is initially contained within a phagosome. Lysosomes containing antimicrobial enzymes and chemicals fuse with the phagosome to create a phagolysosome, where degradation of the pathogen for antigen processing begins. Proteases (protein-degrading) are especially important in antigen processing because only protein antigen epitopes are presented to T cells by MHC II ([\[link\]](#)).

APCs do not present all possible epitopes to T cells; only a selection of the most antigenic or immunodominant epitopes are presented. The mechanism by which epitopes are selected for processing and presentation by an APC is complicated and not well understood; however, once the most antigenic, immunodominant epitopes have been processed, they associate within the antigen-binding cleft of MHC II molecules and are translocated to the cell surface of the dendritic cell for presentation to T cells.



A dendritic cell phagocytoses a bacterial cell and brings it into a phagosome. Lysosomes fuse with the phagosome to create a phagolysosome, where antimicrobial chemicals and enzymes degrade the bacterial cell. Proteases process bacterial antigens, and the most antigenic epitopes are selected and presented on the cell's surface in conjunction with MHC II molecules. T cells recognize the presented antigens and are thus activated.

Note:

- What are the three kinds of APCs?
- What role do MHC II molecules play in antigen presentation?
- What is the role of antigen presentation in adaptive immunity?

Antigen Presentation with MHC I Molecules

MHC I molecules, found on all normal, healthy, nucleated cells, signal to the immune system that the cell is a normal “self” cell. In a healthy cell, proteins normally found in the cytoplasm are degraded by proteasomes (enzyme complexes responsible for degradation and processing of proteins) and processed into self-antigen epitopes; these self-antigen epitopes bind within the MHC I antigen-binding cleft and are then presented on the cell surface. Immune cells, such as NK cells, recognize these self-antigens and do not target the cell for destruction. However, if a cell becomes infected with an intracellular pathogen (e.g., a virus), protein antigens specific to the pathogen are processed in the proteasomes and bind with MHC I molecules for presentation on the cell surface. This presentation of pathogen-specific antigens with MHC I signals that the infected cell must be targeted for destruction along with the pathogen.

Before elimination of infected cells can begin, APCs must first activate the T cells involved in cellular immunity. If an intracellular pathogen directly infects the cytoplasm of an APC, then the processing and presentation of antigens can occur as described (in proteasomes and on the cell surface with MHC I). However, if the intracellular pathogen does not directly infect APCs, an alternative strategy called **cross-presentation** is utilized. In cross-presentation, antigens are brought into the APC by mechanisms normally leading to presentation with MHC II (i.e., through phagocytosis), but the antigen is presented on an MHC I molecule for CD8 T cells. The exact mechanisms by which cross-presentation occur are not yet well understood, but it appears that cross-presentation is primarily a function of dendritic cells and not macrophages or B cells.

Note:

- Compare and contrast antigen processing and presentation associated with MHC I and MHC II molecules.
- What is cross-presentation, and when is it likely to occur?

Key Concepts and Summary

- **Major histocompatibility complex (MHC)** is a collection of genes coding for glycoprotein molecules expressed on the surface of all nucleated cells.
- **MHC I** molecules are expressed on all nucleated cells and are essential for presentation of normal “self” antigens. Cells that become infected by intracellular pathogens can present foreign antigens on MHC I as well, marking the infected cell for destruction.
- **MHC II** molecules are expressed only on the surface of **antigen-presenting cells** (macrophages, dendritic cells, and B cells). Antigen presentation with MHC II is essential for the activation of T cells.
- **Antigen-presenting cells (APCs)** primarily ingest pathogens by phagocytosis, destroy them in the phagolysosomes, process the protein antigens, and select the most antigenic/immunodominant epitopes with MHC II for presentation to T cells.
- **Cross-presentation** is a mechanism of antigen presentation and T-cell activation used by dendritic cells not directly infected by the pathogen; it involves phagocytosis of the pathogen but presentation on MHC I rather than MHC II.

Multiple Choice

Exercise:

Problem: MHC I molecules present

- a. processed foreign antigens from proteasomes.

-
- b. processed self-antigens from phagolysosome.
 - c. antibodies.
 - d. T cell antigens.

Solution:

A

Exercise:

Problem: MHC II molecules present

- a. processed self-antigens from proteasomes.
- b. processed foreign antigens from phagolysosomes.
- c. antibodies.
- d. T cell receptors.

Solution:

B

Exercise:

Problem:

Which type of antigen-presenting molecule is found on all nucleated cells?

- a. MHC II
- b. MHC I
- c. antibodies
- d. B-cell receptors

Solution:

B

Exercise:

Problem:

Which type of antigen-presenting molecule is found only on macrophages, dendritic cells, and B cells?

- a. MHC I
 - b. MHC II
 - c. T-cell receptors
 - d. B-cell receptors
-

Solution:

B

Fill in the Blank

Exercise:

Problem: MHC molecules are used for antigen _____ to T cells.

Solution:

presentation

Exercise:

Problem:

MHC II molecules are made up of two subunits (α and β) of approximately equal size, whereas MHC I molecules consist of a larger α subunit and a smaller subunit called _____.

Solution:

β_2 microglobulin

Critical Thinking

Exercise:

Problem:

Which mechanism of antigen presentation would be used to present antigens from a cell infected with a virus?

Exercise:

Problem:

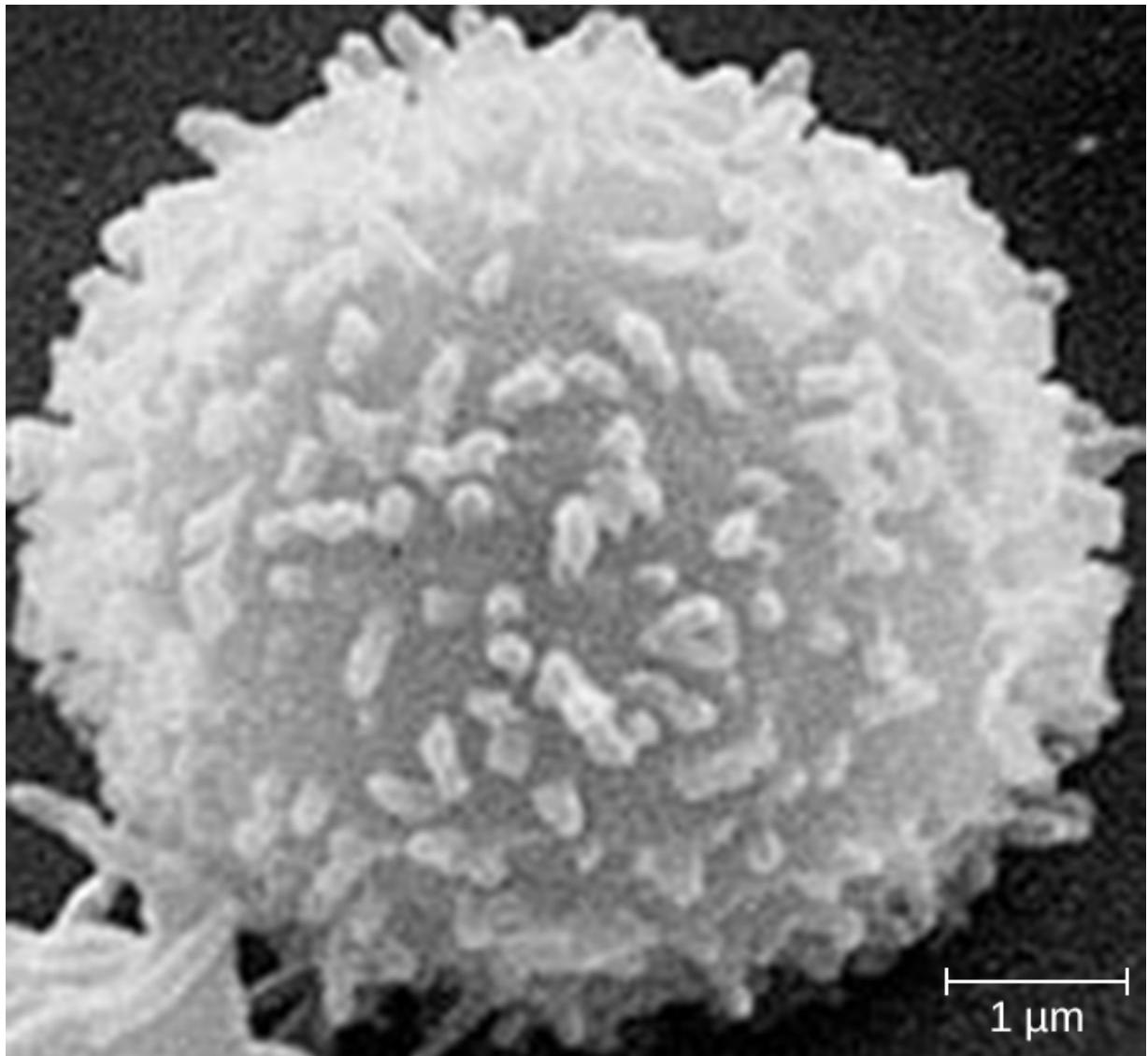
Which pathway of antigen presentation would be used to present antigens from an extracellular bacterial infection?

T Lymphocytes and Cellular Immunity

LEARNING OBJECTIVES

- Describe the process of T-cell maturation and thymic selection
- Explain the genetic events that lead to diversity of T-cell receptors
- Compare and contrast the various classes and subtypes of T cells in terms of activation and function
- Explain the mechanism by which superantigens effect unregulated T-cell activation

As explained in [Overview of Specific Adaptive Immunity](#), the antibodies involved in humoral immunity often bind pathogens and toxins before they can attach to and invade host cells. Thus, humoral immunity is primarily concerned with fighting pathogens in extracellular spaces. However, pathogens that have already gained entry to host cells are largely protected from the humoral antibody-mediated defenses. Cellular immunity, on the other hand, targets and eliminates intracellular pathogens through the actions of T lymphocytes, or T cells ([\[link\]](#)). T cells also play a more central role in orchestrating the overall adaptive immune response (humoral as well as cellular) along with the cellular defenses of innate immunity.



This scanning electron micrograph shows a T lymphocyte, which is responsible for the cell-mediated immune response. The spike-like membrane structures increase surface area, allowing for greater interaction with other cell types and their signals. (credit: modification of work by NCI)

T Cell Production and Maturation

T cells, like all other white blood cells involved in innate and adaptive immunity, are formed from multipotent hematopoietic stem cells (HSCs) in the bone marrow (see [\[link\]](#)). However, unlike the white blood cells of innate immunity, eventual T cells differentiate first into lymphoid stem cells that then become small, immature lymphocytes, sometimes called lymphoblasts. The first steps of differentiation occur in the red marrow of bones ([\[link\]](#)), after which immature T lymphocytes enter the bloodstream and travel to the thymus for the final steps of maturation ([\[link\]](#)). Once in the thymus, the immature T lymphocytes are referred to as thymocytes.

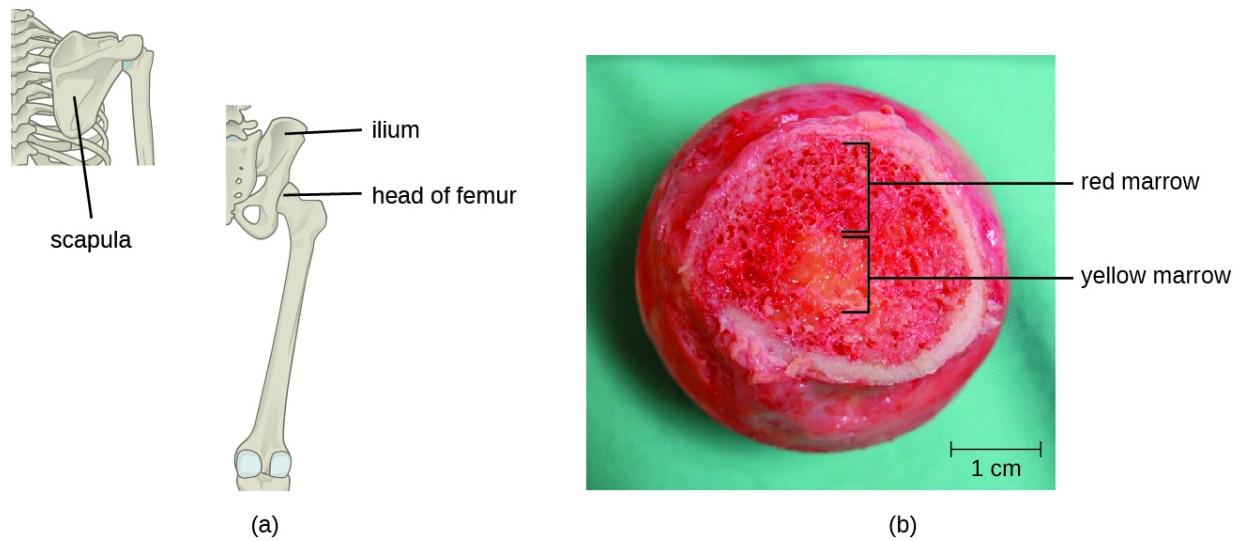
The maturation of thymocytes within the thymus can be divided into three critical steps of positive and negative selection, collectively referred to as **thymic selection**. The first step of thymic selection occurs in the cortex of the thymus and involves the development of a functional T-cell receptor (TCR) that is required for activation by APCs. Thymocytes with defective TCRs are removed by negative selection through the induction of **apoptosis** (programmed controlled cell death). The second step of thymic selection also occurs in the cortex and involves the positive selection of thymocytes that will interact appropriately with MHC molecules. Thymocytes that can interact appropriately with MHC molecules receive a positive stimulation that moves them further through the process of maturation, whereas thymocytes that do not interact appropriately are not stimulated and are eliminated by apoptosis. The third and final step of thymic selection occurs in both the cortex and medulla and involves negative selection to remove self-reacting thymocytes, those that react to self-antigens, by apoptosis. This final step is sometimes referred to as **central tolerance** because it prevents self-reacting T cells from reaching the bloodstream and potentially causing autoimmune disease, which occurs when the immune system attacks healthy “self” cells.

Despite central tolerance, some self-reactive T cells generally escape the thymus and enter the peripheral bloodstream. Therefore, a second line of defense called **peripheral tolerance** is needed to protect against autoimmune disease. Peripheral tolerance involves mechanisms of **anergy** and inhibition of self-reactive T cells by **regulatory T cells**. Anergy refers to a state of nonresponsiveness to antigen stimulation. In the case of self-reactive T cells that escape the thymus, lack of an essential co-stimulatory

signal required for activation causes anergy and prevents autoimmune activation. Regulatory T cells participate in peripheral tolerance by inhibiting the activation and function of self-reactive T cells and by secreting anti-inflammatory cytokines.

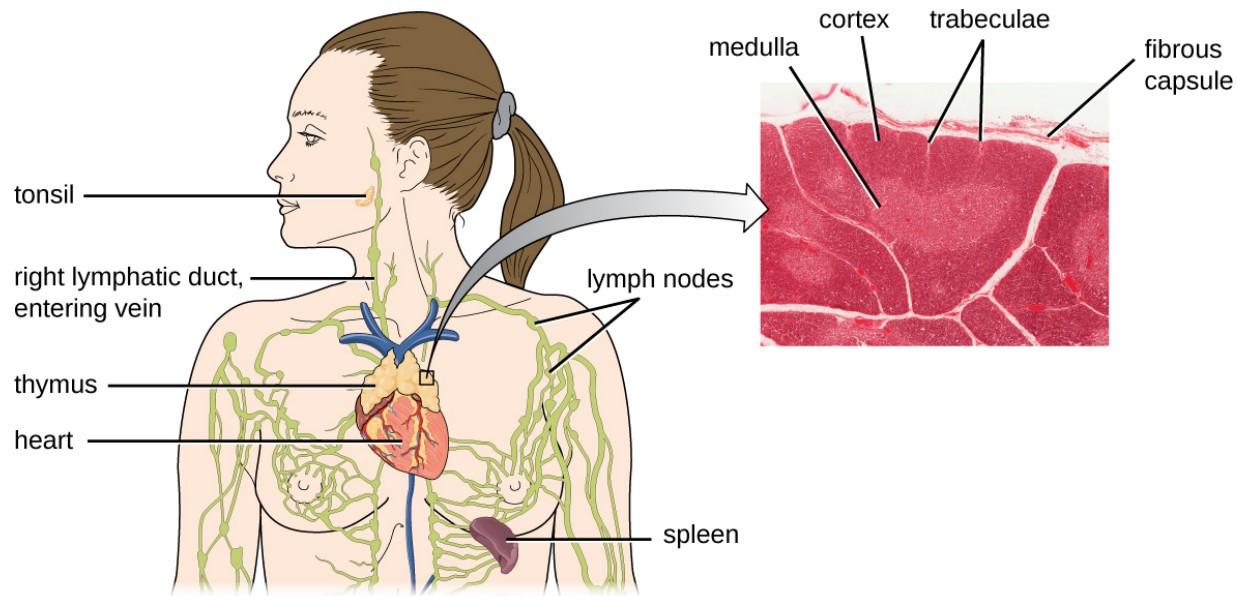
It is not completely understood what events specifically direct maturation of thymocytes into regulatory T cells. Current theories suggest the critical events may occur during the third step of thymic selection, when most self-reactive T cells are eliminated. Regulatory T cells may receive a unique signal that is below the threshold required to target them for negative selection and apoptosis. Consequently, these cells continue to mature and then exit the thymus, armed to inhibit the activation of self-reactive T cells.

It has been estimated that the three steps of thymic selection eliminate 98% of thymocytes. The remaining 2% that exit the thymus migrate through the bloodstream and lymphatic system to sites of secondary lymphoid organs/tissues, such as the lymph nodes, spleen, and tonsils ([\[link\]](#)), where they await activation through the presentation of specific antigens by APCs. Until they are activated, they are known as **mature naïve T cells**.



(a) Red bone marrow can be found in the head of the femur (thighbone) and is also present in the flat bones of the body, such as the ilium and the scapula. (b) Red bone marrow is the site of

production and differentiation of many formed elements of blood, including erythrocytes, leukocytes, and platelets. The yellow bone marrow is populated primarily with adipose cells.



The thymus is a bi-lobed, H-shaped glandular organ that is located just above the heart. It is surrounded by a fibrous capsule of connective tissue. The darkly staining cortex and the lighter staining medulla of individual lobules are clearly visible in the light micrograph of the thymus of a newborn (top right, LM $\times 100$). (credit micrograph: modification of micrograph provided by the Regents of University of Michigan Medical School © 2012)

Note:

- What anatomical sites are involved in T cell production and maturation?
- What are the three steps involved in thymic selection?
- Why are central tolerance and peripheral tolerance important? What do they prevent?

Classes of T Cells

T cells can be categorized into three distinct classes: helper T cells, regulatory T cells, and cytotoxic T cells. These classes are differentiated based on their expression of certain surface molecules, their mode of activation, and their functional roles in adaptive immunity ([\[link\]](#)).

All T cells produce **cluster of differentiation (CD) molecules**, cell surface glycoproteins that can be used to identify and distinguish between the various types of white blood cells. Although T cells can produce a variety of CD molecules, CD4 and CD8 are the two most important used for differentiation of the classes. Helper T cells and regulatory T cells are characterized by the expression of CD4 on their surface, whereas cytotoxic T cells are characterized by the expression of CD8.

Classes of T cells can also be distinguished by the specific MHC molecules and APCs with which they interact for activation. Helper T cells and regulatory T cells can only be activated by APCs presenting antigens associated with MHC II. In contrast, cytotoxic T cells recognize antigens presented in association with MHC I, either by APCs or by nucleated cells infected with an intracellular pathogen.

The different classes of T cells also play different functional roles in the immune system. **Helper T cells** serve as the central orchestrators that help activate and direct functions of humoral and cellular immunity. In addition, helper T cells enhance the pathogen-killing functions of macrophages and NK cells of innate immunity. In contrast, the primary role of regulatory T cells is to prevent undesirable and potentially damaging immune responses. Their role in peripheral tolerance, for example, protects against autoimmune

disorders, as discussed earlier. Finally, **cytotoxic T cells** are the primary effector cells for cellular immunity. They recognize and target cells that have been infected by intracellular pathogens, destroying infected cells along with the pathogens inside.

Classes of T Cells			
Class	Surface CD Molecules	Activation	Functions
Helper T cells	CD4	APCs presenting antigens associated with MHC II	Orchestrate humoral and cellular immunity
			Involved in the activation of macrophages and NK cells
Regulatory T cells	CD4	APCs presenting antigens associated with MHC II	Involved in peripheral tolerance and prevention of autoimmune responses

Classes of T Cells			
Class	Surface CD Molecules	Activation	Functions
Cytotoxic T cells	CD8	APCs or infected nucleated cells presenting antigens associated with MHC I	Destroy cells infected with intracellular pathogens

Note:

- What are the unique functions of the three classes of T cells?
- Which T cells can be activated by antigens presented by cells other than APCs?

T-Cell Receptors

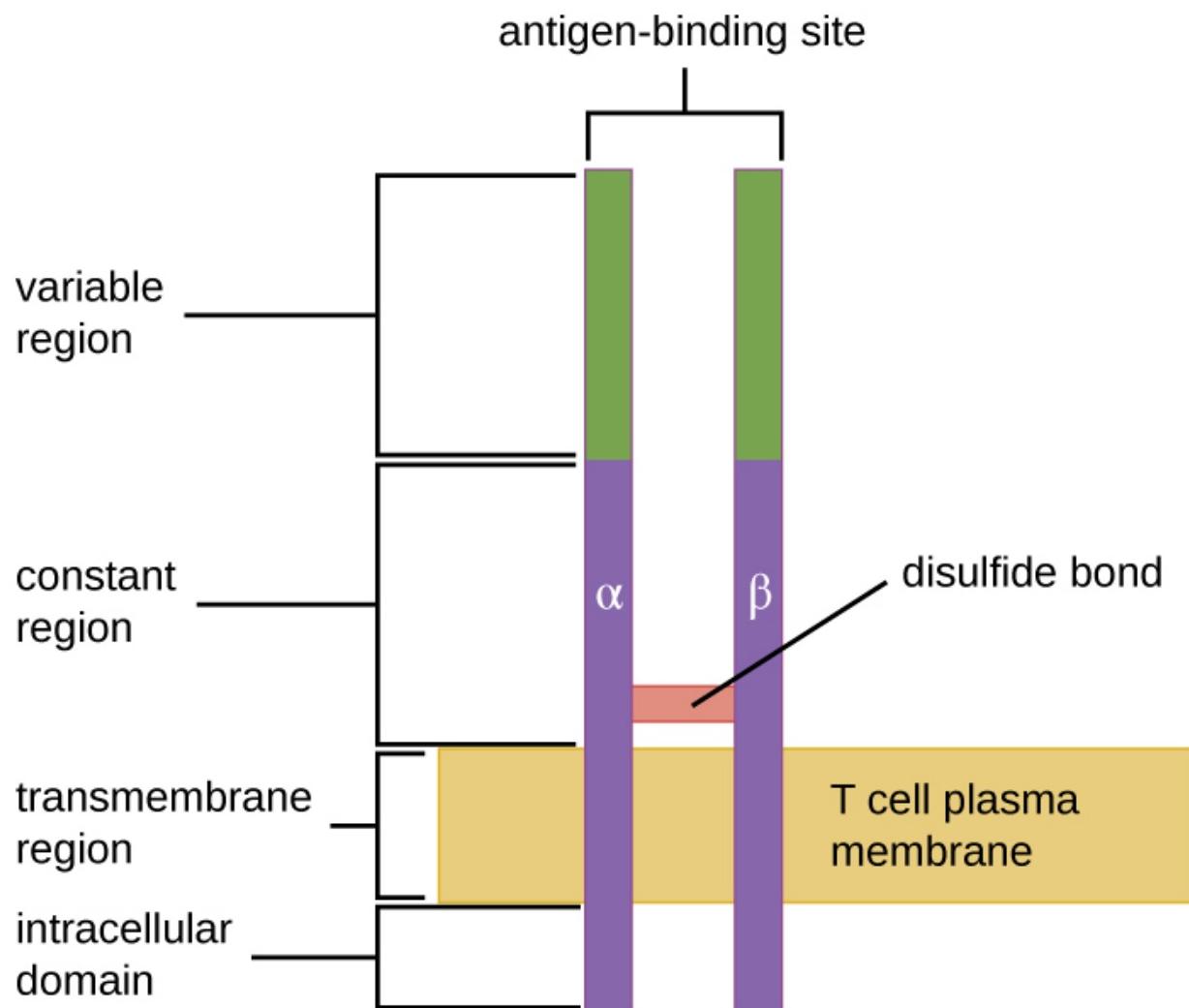
For both helper T cells and cytotoxic T cells, activation is a complex process that requires the interactions of multiple molecules and exposure to cytokines. The **T-cell receptor (TCR)** is involved in the first step of pathogen epitope recognition during the activation process.

The TCR comes from the same receptor family as the antibodies IgD and IgM, the antigen receptors on the B cell membrane surface, and thus shares common structural elements. Similar to antibodies, the TCR has a variable region and a constant region, and the variable region provides the antigen-binding site ([\[link\]](#)). However, the structure of TCR is smaller and less

complex than the immunoglobulin molecules ([\[link\]](#)). Whereas immunoglobulins have four peptide chains and Y-shaped structures, the TCR consists of just two peptide chains (α and β chains), both of which span the cytoplasmic membrane of the T cell.

TCRs are epitope-specific, and it has been estimated that 25 million T cells with unique epitope-binding TCRs are required to protect an individual against a wide range of microbial pathogens. Because the human genome only contains about 25,000 genes, we know that each specific TCR cannot be encoded by its own set of genes. This raises the question of how such a vast population of T cells with millions of specific TCRs can be achieved. The answer is a process called genetic rearrangement, which occurs in the thymus during the first step of thymic selection.

The genes that code for the variable regions of the TCR are divided into distinct gene segments called variable (V), diversity (D), and joining (J) segments. The genes segments associated with the α chain of the TCR consist 70 or more different V_α segments and 61 different J_α segments. The gene segments associated with the β chain of the TCR consist of 52 different V_β segments, two different D_β segments, and 13 different J_β segments. During the development of the functional TCR in the thymus, genetic rearrangement in a T cell brings together one V_α segment and one J_α segment to code for the variable region of the α chain. Similarly, genetic rearrangement brings one of the V_β segments together with one of the D_β segments and one of the J_β segments to code for the variable region of the β chain. All the possible combinations of rearrangements between different segments of V, D, and J provide the genetic diversity required to produce millions of TCRs with unique epitope-specific variable regions.



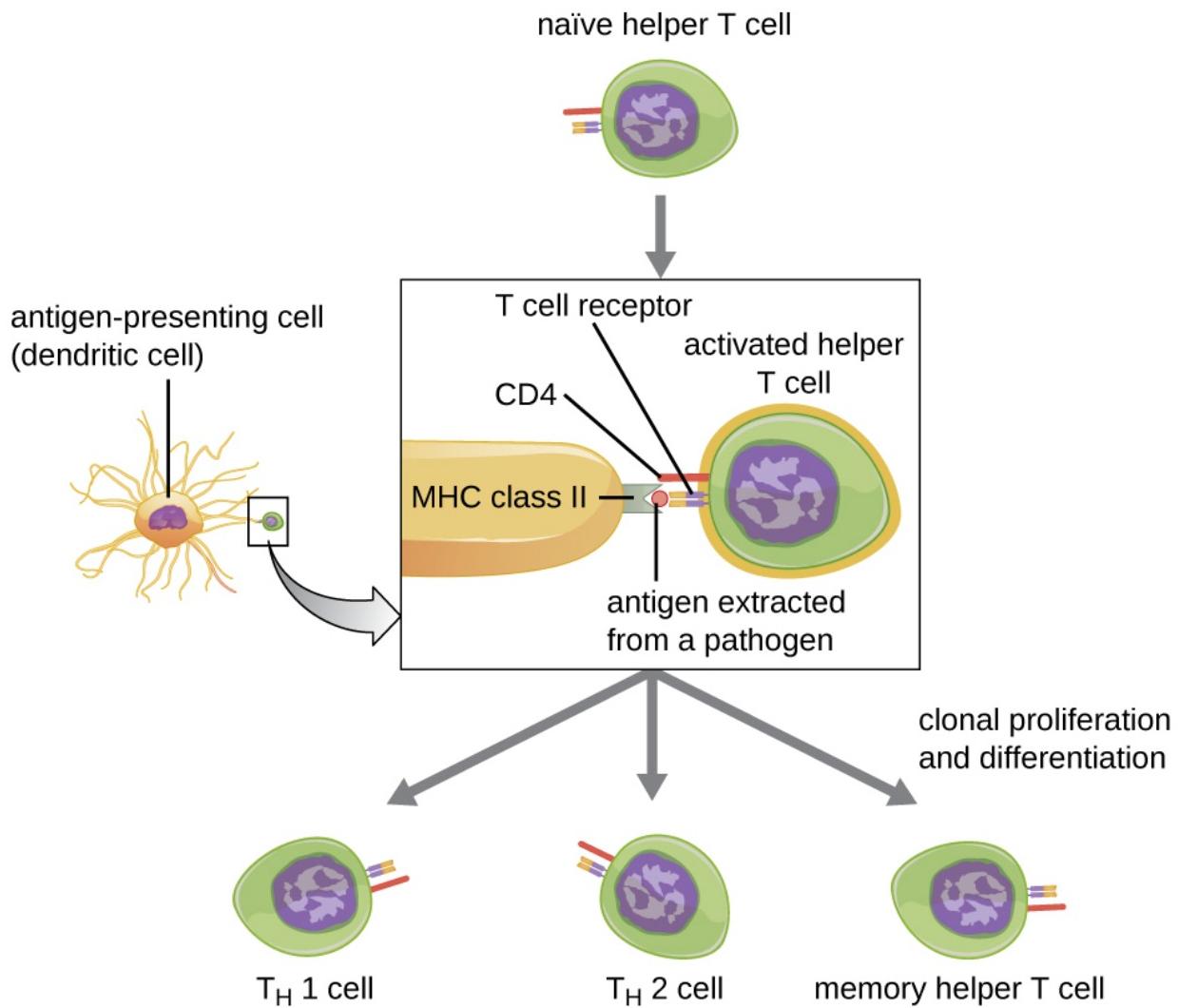
A T-cell receptor spans the cytoplasmic membrane and projects variable binding regions into the extracellular space to bind processed antigens associated with MHC I or MHC II molecules.

Note:

- What are the similarities and differences between TCRs and immunoglobulins?
- What process is used to provide millions of unique TCR binding sites?

Activation and Differentiation of Helper T Cells

Helper T cells can only be activated by APCs presenting processed foreign epitopes in association with MHC II. The first step in the activation process is TCR recognition of the specific foreign epitope presented within the MHC II antigen-binding cleft. The second step involves the interaction of CD4 on the helper T cell with a region of the MHC II molecule separate from the antigen-binding cleft. This second interaction anchors the MHC II-TCR complex and ensures that the helper T cell is recognizing both the foreign (“nonself”) epitope and “self” antigen of the APC; both recognitions are required for activation of the cell. In the third step, the APC and T cell secrete cytokines that activate the helper T cell. The activated helper T cell then proliferates, dividing by mitosis to produce clonal naïve helper T cells that differentiate into subtypes with different functions ([\[link\]](#)).



This illustration depicts the activation of a naïve (unactivated) helper T cell by an antigen-presenting cell and the subsequent proliferation and differentiation of the activated T cell into different subtypes.

Activated helper T cells can differentiate into one of four distinct subtypes, summarized in [\[link\]](#). The differentiation process is directed by APC-secreted cytokines. Depending on which APC-secreted cytokines interact with an activated helper T cell, the cell may differentiate into a T helper 1 ($T_H 1$) cell, a T helper 2 ($T_H 2$) cell, or a memory helper T cell. The two types of helper T cells are relatively short-lived **effector cells**, meaning that they perform various functions of the immediate immune response. In

contrast, **memory helper T cells** are relatively long lived; they are programmed to “remember” a specific antigen or epitope in order to mount a rapid, strong, secondary response to subsequent exposures.

T_H1 cells secrete their own cytokines that are involved in stimulating and orchestrating other cells involved in adaptive and innate immunity. For example, they stimulate cytotoxic T cells, enhancing their killing of infected cells and promoting differentiation into memory cytotoxic T cells. T_H1 cells also stimulate macrophages and neutrophils to become more effective in their killing of intracellular bacteria. They can also stimulate NK cells to become more effective at killing target cells.

T_H2 cells play an important role in orchestrating the humoral immune response through their secretion of cytokines that activate B cells and direct B cell differentiation and antibody production. Various cytokines produced by T_H2 cells orchestrate antibody class switching, which allows B cells to switch between the production of IgM, IgG, IgA, and IgE as needed to carry out specific antibody functions and to provide pathogen-specific humoral immune responses.

A third subtype of helper T cells called **T_H17 cells** was discovered through observations that immunity to some infections is not associated with T_H1 or T_H2 cells. T_H17 cells and the cytokines they produce appear to be specifically responsible for the body’s defense against chronic mucocutaneous infections. Patients who lack sufficient T_H17 cells in the mucosa (e.g., HIV patients) may be more susceptible to bacteremia and gastrointestinal infections. [\[footnote\]](#)

Blaschitz C., Raffatellu M. “Th17 cytokines and the gut mucosal barrier.” J Clin Immunol. 2010 Mar; 30(2):196-203. doi: 10.1007/s10875-010-9368-7.

Subtypes of Helper T Cells

Subtypes of Helper T Cells

Subtype	Functions
T_{H1} cells	Stimulate cytotoxic T cells and produce memory cytotoxic T cells
	Stimulate macrophages and neutrophils (PMNs) for more effective intracellular killing of pathogens
	Stimulate NK cells to kill more effectively
T_{H2} cells	Stimulate B cell activation and differentiation into plasma cells and memory B cells
	Direct antibody class switching in B cells
T_{H17} cells	Stimulate immunity to specific infections such as chronic mucocutaneous infections
Memory helper T cells	“Remember” a specific pathogen and mount a strong, rapid secondary response upon re-exposure

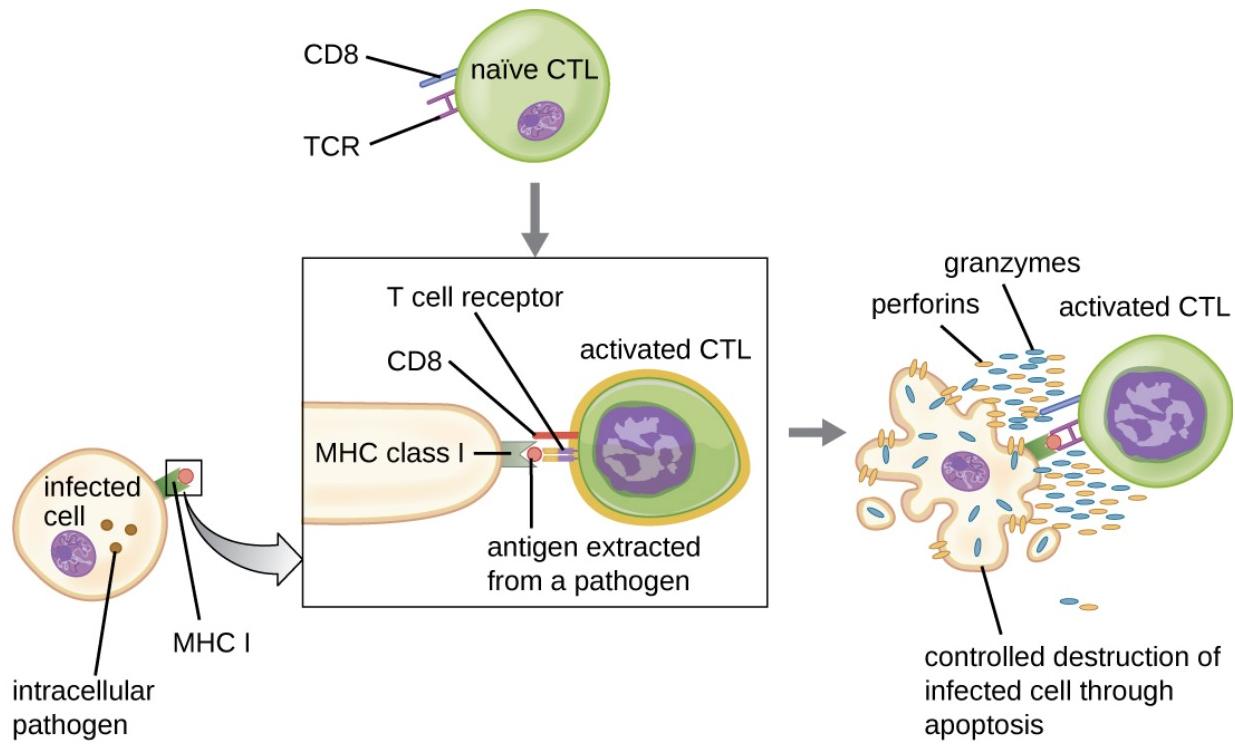
Activation and Differentiation of Cytotoxic T Cells

Cytotoxic T cells (also referred to as cytotoxic T lymphocytes, or CTLs) are activated by APCs in a three-step process similar to that of helper T cells. The key difference is that the activation of cytotoxic T cells involves recognition of an antigen presented with MHC I (as opposed to MHC II) and interaction of CD8 (as opposed to CD4) with the receptor complex. After the successful co-recognition of foreign epitope and self-antigen, the production of cytokines by the APC and the cytotoxic T cell activate clonal proliferation and differentiation. Activated cytotoxic T cells can

differentiate into effector cytotoxic T cells that target pathogens for destruction or memory cells that are ready to respond to subsequent exposures.

As noted, proliferation and differentiation of cytotoxic T cells is also stimulated by cytokines secreted from $T_{H}1$ cells activated by the same foreign epitope. The co-stimulation that comes from these $T_{H}1$ cells is provided by secreted cytokines. Although it is possible for activation of cytotoxic T cells to occur without stimulation from $T_{H}1$ cells, the activation is not as effective or long-lasting.

Once activated, cytotoxic T cells serve as the effector cells of cellular immunity, recognizing and kill cells infected with intracellular pathogens through a mechanism very similar to that of NK cells. However, whereas NK cells recognize nonspecific signals of cell stress or abnormality, cytotoxic T cells recognize infected cells through antigen presentation of pathogen-specific epitopes associated with MHC I. Once an infected cell is recognized, the TCR of the cytotoxic T cell binds to the epitope and releases perforin and granzymes that destroy the infected cell ([\[link\]](#)). Perforin is a protein that creates pores in the target cell, and **granzymes** are proteases that enter the pores and induce apoptosis. This mechanism of programmed cell death is a controlled and efficient means of destroying and removing infected cells without releasing the pathogens inside to infect neighboring cells, as might occur if the infected cells were simply lysed.



This figure illustrates the activation of a naïve (unactivated) cytotoxic T cell (CTL) by an antigen-presenting MHC I molecule on an infected body cell. Once activated, the CTL releases perforin and granzymes that invade the infected cell and induce controlled cell death, or apoptosis.

Note:



In this [video](#), you can see a cytotoxic T cell inducing apoptosis in a target cell.

Note:

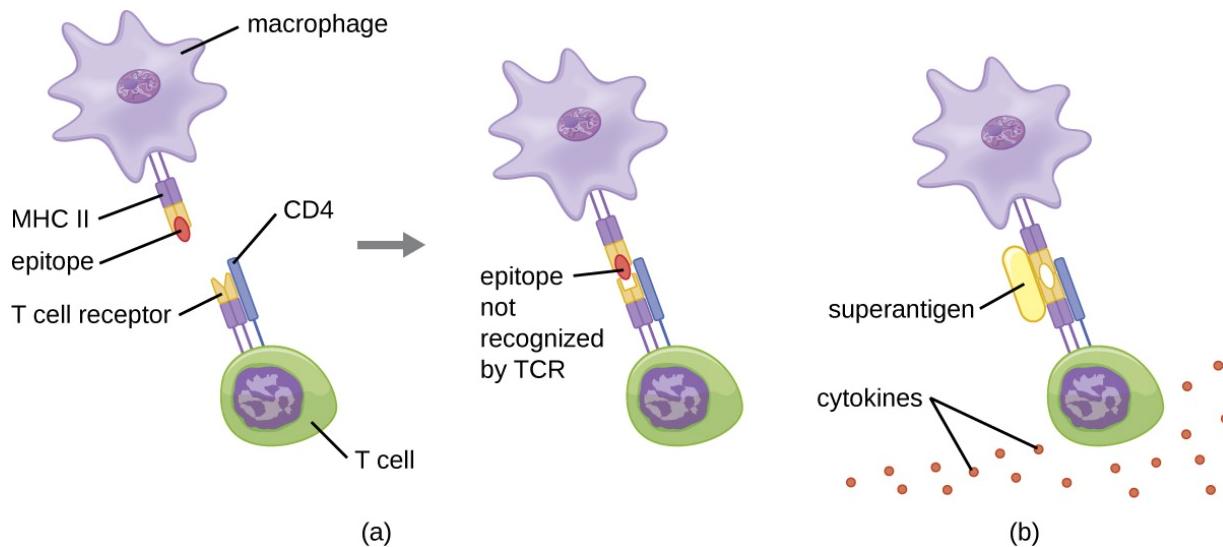
- Compare and contrast the activation of helper T cells and cytotoxic T cells.
- What are the different functions of helper T cell subtypes?
- What is the mechanism of CTL-mediated destruction of infected cells?

Superantigens and Unregulated Activation of T Cells

When T cell activation is controlled and regulated, the result is a protective response that is effective in combating infections. However, if T cell activation is unregulated and excessive, the result can be a life-threatening. Certain bacterial and viral pathogens produce toxins known as superantigens (see [Virulence Factors of Bacterial and Viral Pathogens](#)) that can trigger such an unregulated response. Known bacterial superantigens include toxic shock syndrome toxin (TSST), staphylococcal enterotoxins, streptococcal pyrogenic toxins, streptococcal superantigen, and the streptococcal mitogenic exotoxin. Viruses known to produce superantigens include Epstein-Barr virus (human herpesvirus 4), cytomegalovirus (human herpesvirus 5), and others.

The mechanism of T cell activation by superantigens involves their simultaneous binding to MHC II molecules of APCs and the variable region of the TCR β chain. This binding occurs outside of the antigen-binding cleft of MHC II, so the superantigen will bridge together and activate MHC II and TCR without specific foreign epitope recognition ([\[link\]](#)). The result is an excessive, uncontrolled release of cytokines, often called a **cytokine storm**, which stimulates an excessive inflammatory response. This can lead

to a dangerous decrease in blood pressure, shock, multi-organ failure, and potentially, death.



(a) The macrophage in this figure is presenting a foreign epitope that does not match the TCR of the T cell. Because the T cell does not recognize the epitope, it is not activated. (b) The macrophage in this figure is presenting a superantigen that is not recognized by the TCR of the T cell, yet the superantigen still is able to bridge and bind the MHC II and TCR molecules. This nonspecific, uncontrolled activation of the T cell results in an excessive release of cytokines that activate other T cells and cause excessive inflammation. (credit: modification of work by “Microbiotic”/YouTube)

Note:

- What are examples of superantigens?
- How does a superantigen activate a helper T cell?
- What effect does a superantigen have on a T cell?

Note:**Superantigens**

Melissa, an otherwise healthy 22-year-old woman, is brought to the emergency room by her concerned boyfriend. She complains of a sudden onset of high fever, vomiting, diarrhea, and muscle aches. In her initial interview, she tells the attending physician that she is on hormonal birth control and also is two days into the menstruation portion of her cycle. She is on no other medications and is not abusing any drugs or alcohol. She is not a smoker. She is not diabetic and does not currently have an infection of any kind to her knowledge.

While waiting in the emergency room, Melissa's blood pressure begins to drop dramatically and her mental state deteriorates to general confusion. The physician believes she is likely suffering from toxic shock syndrome (TSS). TSS is caused by the toxin TSST-1, a superantigen associated with *Staphylococcus aureus*, and improper tampon use is a common cause of infections leading to TSS. The superantigen inappropriately stimulates widespread T cell activation and excessive cytokine release, resulting in a massive and systemic inflammatory response that can be fatal.

Vaginal or cervical swabs may be taken to confirm the presence of the microbe, but these tests are not critical to perform based on Melissa's symptoms and medical history. The physician prescribes rehydration, supportive therapy, and antibiotics to stem the bacterial infection. She also prescribes drugs to increase Melissa's blood pressure. Melissa spends three days in the hospital undergoing treatment; in addition, her kidney function is monitored because of the high risk of kidney failure associated with TSS. After 72 hours, Melissa is well enough to be discharged to continue her recovery at home.

- In what way would antibiotic therapy help to combat a superantigen?

Note:**Part 2**

Olivia's swollen lymph nodes, abdomen, and spleen suggest a strong immune response to a systemic infection in progress. In addition, little

Olivia is reluctant to turn her head and appears to be experiencing severe neck pain. The physician orders a complete blood count, blood culture, and lumbar puncture. The cerebrospinal fluid (CSF) obtained appears cloudy and is further evaluated by Gram stain assessment and culturing for potential bacterial pathogens. The complete blood count indicates elevated numbers of white blood cells in Olivia's bloodstream. The white blood cell increases are recorded at 28.5 K/ μ L (normal range: 6.0–17.5 K/ μ L). The neutrophil percentage was recorded as 60% (normal range: 23–45%). Glucose levels in the CSF were registered at 30 mg/100 mL (normal range: 50–80 mg/100 mL). The WBC count in the CSF was 1,163/mm³ (normal range: 5–20/mm³).

- Based on these results, do you have a preliminary diagnosis?
- What is a recommended treatment based on this preliminary diagnosis?

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

Key Concepts and Summary

- Immature T lymphocytes are produced in the red bone marrow and travel to the thymus for maturation.
- **Thymic selection** is a three-step process of negative and positive selection that determines which T cells will mature and exit the thymus into the peripheral bloodstream.
- **Central tolerance** involves negative selection of self-reactive T cells in the thymus, and **peripheral tolerance** involves **anergy** and **regulatory T cells** that prevent self-reactive immune responses and autoimmunity.
- The **TCR** is similar in structure to immunoglobulins, but less complex. Millions of unique epitope-binding TCRs are encoded through a process of genetic rearrangement of V, D, and J gene segments.
- T cells can be divided into three classes—**helper T cells**, **cytotoxic T cells**, and **regulatory T cells**—based on their expression of CD4 or

CD8, the MHC molecules with which they interact for activation, and their respective functions.

- Activated helper T cells differentiate into **T_H1**, **T_H2**, **T_H17**, or **memory T cell subtypes**. Differentiation is directed by the specific cytokines to which they are exposed. T_H1, T_H2, and T_H17 perform different functions related to stimulation of adaptive and innate immune defenses. Memory T cells are long-lived cells that can respond quickly to secondary exposures.
- Once activated, cytotoxic T cells target and kill cells infected with intracellular pathogens. Killing requires recognition of specific pathogen epitopes presented on the cell surface using MHC I molecules. Killing is mediated by **perforin** and **granzymes** that induce apoptosis.
- **Superantigens** are bacterial or viral proteins that cause a nonspecific activation of helper T cells, leading to an excessive release of cytokines (**cytokine storm**) and a systemic, potentially fatal inflammatory response.

Multiple Choice

Exercise:

Problem: What is a superantigen?

- a protein that is highly efficient at stimulating a single type of productive and specific T cell response
- a protein produced by antigen-presenting cells to enhance their presentation capabilities
- a protein produced by T cells as a way of increasing the antigen activation they receive from antigen-presenting cells
- a protein that activates T cells in a nonspecific and uncontrolled manner

Solution:

D

Exercise:

Problem: To what does the TCR of a helper T cell bind?

- a. antigens presented with MHC I molecules
 - b. antigens presented with MHC II molecules
 - c. free antigen in a soluble form
 - d. haptens only
-

Solution:

B

Exercise:

Problem:

Cytotoxic T cells will bind with their TCR to which of the following?

- a. antigens presented with MHC I molecules
 - b. antigens presented with MHC II molecules
 - c. free antigen in a soluble form
 - d. haptens only
-

Solution:

A

Exercise:

Problem:

A _____ molecule is a glycoprotein used to identify and distinguish white blood cells.

- a. T-cell receptor
- b. B-cell receptor
- c. MHC I

d. cluster of differentiation

Solution:

D

Exercise:

Problem:

Name the T helper cell subset involved in antibody production.

- a. T_H1
 - b. T_H2
 - c. T_H17
 - d. CTL
-

Solution:

B

Fill in the Blank

Exercise:

Problem:

A _____ T cell will become activated by presentation of foreign antigen associated with an MHC I molecule.

Solution:

cytotoxic

Exercise:

Problem:

A _____ T cell will become activated by presentation of foreign antigen in association with an MHC II molecule.

Solution:

helper

Exercise:**Problem:**

A TCR is a protein dimer embedded in the plasma membrane of a T cell. The _____ region of each of the two protein chains is what gives it the capability to bind to a presented antigen.

Solution:

variable

Exercise:**Problem:**

Peripheral tolerance mechanisms function on T cells after they mature and exit the _____.

Solution:

thymus

Exercise:**Problem:**

Both _____ and effector T cells are produced during differentiation of activated T cells.

Solution:

memory

Short Answer

Exercise:

Problem:

What is the basic difference in effector function between helper and cytotoxic T cells?

Exercise:

Problem:

What necessary interactions are required for activation of helper T cells and activation/effector function of cytotoxic T cells?

B Lymphocytes and Humoral Immunity

LEARNING OBJECTIVES

- Describe the production and maturation of B cells
- Compare the structure of B-cell receptors and T-cell receptors
- Compare T-dependent and T-independent activation of B cells
- Compare the primary and secondary antibody responses

Humoral immunity refers to mechanisms of the adaptive immune defenses that are mediated by antibodies secreted by B lymphocytes, or B cells. This section will focus on B cells and discuss their production and maturation, receptors, and mechanisms of activation.

B Cell Production and Maturation

Like T cells, B cells are formed from multipotent hematopoietic stem cells (HSCs) in the bone marrow and follow a pathway through lymphoid stem cell and lymphoblast (see [[link](#)]). Unlike T cells, however, lymphoblasts destined to become B cells do not leave the bone marrow and travel to the thymus for maturation. Rather, eventual B cells continue to mature in the bone marrow.

The first step of B cell maturation is an assessment of the functionality of their antigen-binding receptors. This occurs through positive selection for B cells with normal functional receptors. A mechanism of negative selection is then used to eliminate self-reacting B cells and minimize the risk of autoimmunity. Negative selection of self-reacting B cells can involve elimination by apoptosis, editing or modification of the receptors so they

are no longer self-reactive, or induction of anergy in the B cell. Immature B cells that pass the selection in the bone marrow then travel to the spleen for their final stages of maturation. There they become **naïve mature B cells**, i.e., mature B cells that have not yet been activated.

Note:

- Compare the maturation of B cells with the maturation of T cells.

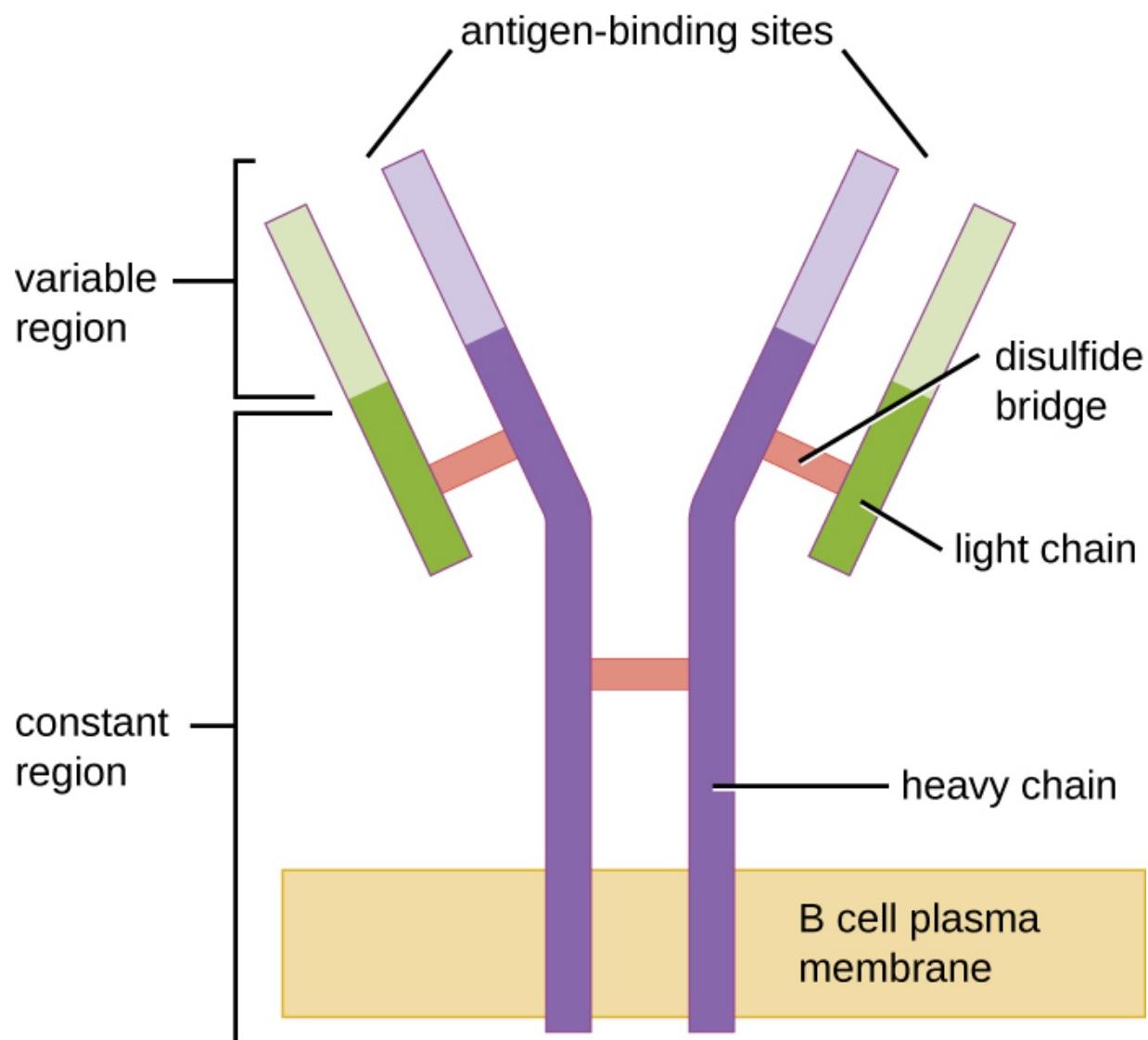
B-Cell Receptors

Like T cells, B cells possess antigen-specific receptors with diverse specificities. Although they rely on T cells for optimum function, B cells can be activated without help from T cells. **B-cell receptors (BCRs)** for naïve mature B cells are membrane-bound monomeric forms of IgD and IgM. They have two identical heavy chains and two identical light chains connected by disulfide bonds into a basic “Y” shape ([\[link\]](#)). The trunk of the Y-shaped molecule, the constant region of the two heavy chains, spans the B cell membrane. The two antigen-binding sites exposed to the exterior of the B cell are involved in the binding of specific pathogen epitopes to initiate the activation process. It is estimated that each naïve mature B cell has upwards of 100,000 BCRs on its membrane, and each of these BCRs has an identical epitope-binding specificity.

In order to be prepared to react to a wide range of microbial epitopes, B cells, like T cells, use genetic rearrangement of hundreds of gene segments to provide the necessary diversity of receptor specificities. The variable region of the BCR heavy chain is made up of V, D, and J segments, similar to the β chain of the TCR. The variable region of the BCR light chain is made up of V and J segments, similar to the α chain of the TCR. Genetic rearrangement of all possible combinations of V-J-D (heavy chain) and V-J (light chain) provides for millions of unique antigen-binding sites for the BCR and for the antibodies secreted after activation.

One important difference between BCRs and TCRs is the way they can interact with antigenic epitopes. Whereas TCRs can only interact with antigenic epitopes that are presented within the antigen-binding cleft of MHC I or MHC II, BCRs do not require antigen presentation with MHC; they can interact with epitopes on free antigens or with epitopes displayed on the surface of intact pathogens. Another important difference is that TCRs only recognize protein epitopes, whereas BCRs can recognize epitopes associated with different molecular classes (e.g., proteins, polysaccharides, lipopolysaccharides).

Activation of B cells occurs through different mechanisms depending on the molecular class of the antigen. Activation of a B cell by a protein antigen requires the B cell to function as an APC, presenting the protein epitopes with MHC II to helper T cells. Because of their dependence on T cells for activation of B cells, protein antigens are classified as **T-dependent antigens**. In contrast, polysaccharides, lipopolysaccharides, and other nonprotein antigens are considered **T-independent antigens** because they can activate B cells without antigen processing and presentation to T cells.



B-cell receptors are embedded in the membranes of B cells. The variable regions of all of the receptors on a single cell bind the same specific antigen.

Note:

- What types of molecules serve as the BCR?

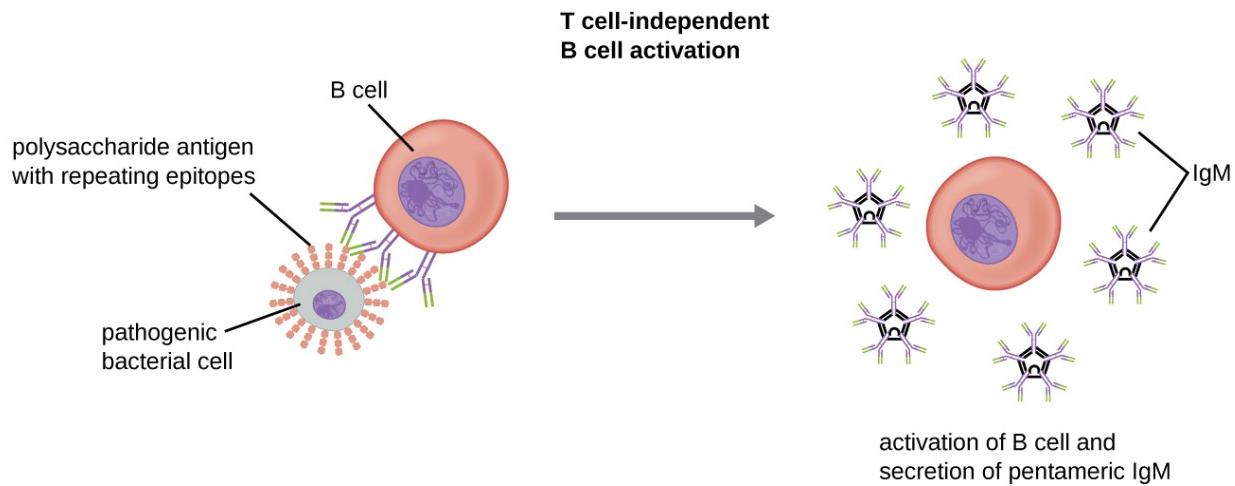
- What are the differences between TCRs and BCRs with respect to antigen recognition?
- Which molecule classes are T-dependent antigens and which are T-independent antigens?

T Cell-Independent Activation of B cells

Activation of B cells without the cooperation of helper T cells is referred to as T cell-independent activation and occurs when BCRs interact with T-independent antigens. T-independent antigens (e.g., polysaccharide capsules, lipopolysaccharide) have repetitive epitope units within their structure, and this repetition allows for the cross-linkage of multiple BCRs, providing the first signal for activation ([\[link\]](#)). Because T cells are not involved, the second signal has to come from other sources, such as interactions of toll-like receptors with PAMPs or interactions with factors from the complement system.

Once a B cell is activated, it undergoes clonal proliferation and daughter cells differentiate into plasma cells. **Plasma cells** are antibody factories that secrete large quantities of antibodies. After differentiation, the surface BCRs disappear and the plasma cell secretes pentameric IgM molecules that have the same antigen specificity as the BCRs ([\[link\]](#)).

The T cell-independent response is short-lived and does not result in the production of memory B cells. Thus it will not result in a secondary response to subsequent exposures to T-independent antigens.



T-independent antigens have repeating epitopes that can induce B cell recognition and activation without involvement from T cells. A second signal, such as interaction of TLRs with PAMPs (not shown), is also required for activation of the B cell. Once activated, the B cell proliferates and differentiates into antibody-secreting plasma cells.

Note:

- What are the two signals required for T cell-independent activation of B cells?
- What is the function of a plasma cell?

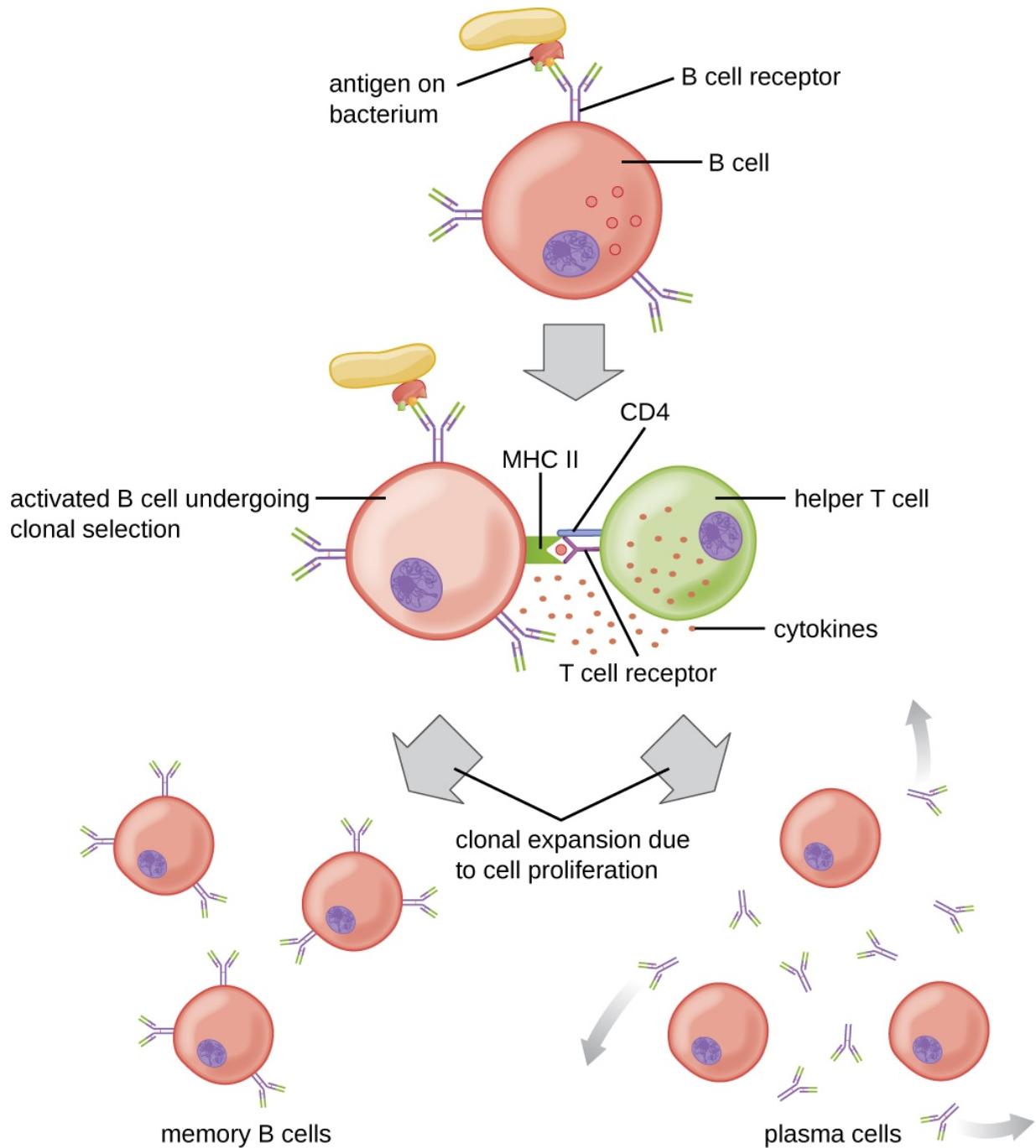
T Cell-Dependent Activation of B cells

T cell-dependent activation of B cells is more complex than T cell-independent activation, but the resulting immune response is stronger and develops memory. T cell-dependent activation can occur either in response to free protein antigens or to protein antigens associated with an intact pathogen. Interaction between the BCRs on a naïve mature B cell and a free

protein antigen stimulate internalization of the antigen, whereas interaction with antigens associated with an intact pathogen initiates the extraction of the antigen from the pathogen before internalization. Once internalized inside the B cell, the protein antigen is processed and presented with MHC II. The presented antigen is then recognized by helper T cells specific to the same antigen. The TCR of the helper T cell recognizes the foreign antigen, and the T cell's CD4 molecule interacts with MHC II on the B cell. The coordination between B cells and helper T cells that are specific to the same antigen is referred to as **linked recognition**.

Once activated by linked recognition, T_{H2} cells produce and secrete cytokines that activate the B cell and cause proliferation into clonal daughter cells. After several rounds of proliferation, additional cytokines provided by the T_{H2} cells stimulate the differentiation of activated B cell clones into **memory B cells**, which will quickly respond to subsequent exposures to the same protein epitope, and plasma cells that lose their membrane BCRs and initially secrete pentameric IgM ([\[link\]](#)).

After initial secretion of IgM, cytokines secreted by T_{H2} cells stimulate the plasma cells to switch from IgM production to production of IgG, IgA, or IgE. This process, called **class switching** or isotype switching, allows plasma cells cloned from the same activated B cell to produce a variety of antibody classes with the same epitope specificity. Class switching is accomplished by genetic rearrangement of gene segments encoding the constant region, which determines an antibody's class. The variable region is not changed, so the new class of antibody retains the original epitope specificity.



In T cell-dependent activation of B cells, the B cell recognizes and internalizes an antigen and presents it to a helper T cell that is specific to the same antigen. The helper T cell interacts with the antigen presented by the B cell, which activates the T cell and stimulates the release of cytokines that then activate the B cell. Activation of the B

cell triggers proliferation and differentiation into B cells and plasma cells.

Note:

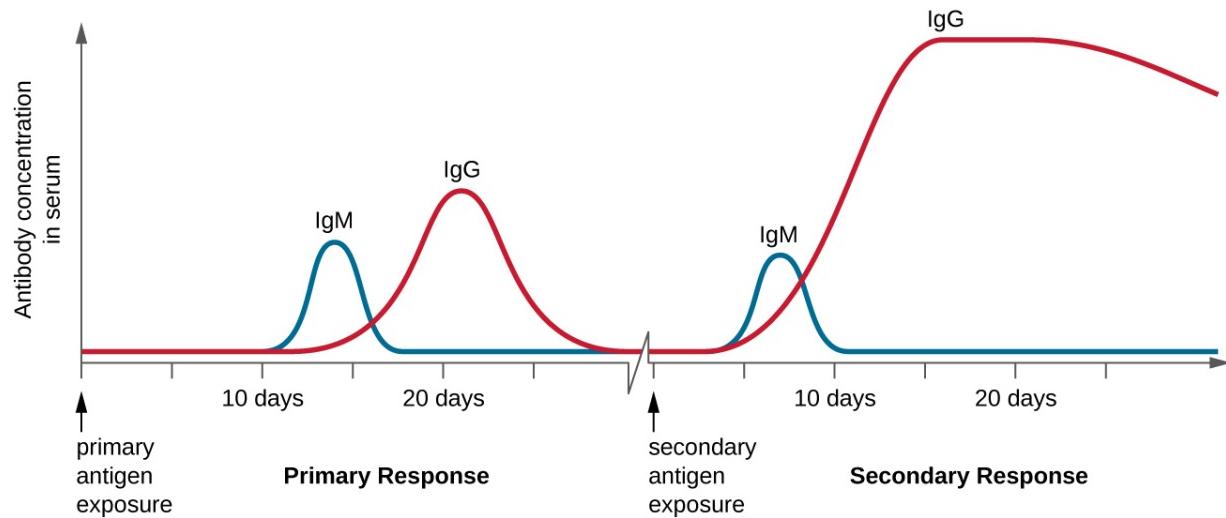
- What steps are required for T cell-dependent activation of B cells?
- What is antibody class switching and why is it important?

Primary and Secondary Responses

T cell-dependent activation of B cells plays an important role in both the primary and secondary responses associated with adaptive immunity. With the first exposure to a protein antigen, a T cell-dependent primary antibody response occurs. The initial stage of the primary response is a **lag period**, or latent period, of approximately 10 days, during which no antibody can be detected in serum. This lag period is the time required for all of the steps of the primary response, including naïve mature B cell binding of antigen with BCRs, antigen processing and presentation, helper T cell activation, B cell activation, and clonal proliferation. The end of the lag period is characterized by a rise in IgM levels in the serum, as T_{H2} cells stimulate B cell differentiation into plasma cells. IgM levels reach their peak around 14 days after primary antigen exposure; at about this same time, T_{H2} stimulates antibody class switching, and IgM levels in serum begin to decline. Meanwhile, levels of IgG increase until they reach a peak about three weeks into the primary response ([\[link\]](#)).

During the primary response, some of the cloned B cells are differentiated into memory B cells programmed to respond to subsequent exposures. This secondary response occurs more quickly and forcefully than the primary response. The lag period is decreased to only a few days and the production of IgG is significantly higher than observed for the primary response ([\[link\]](#)). In addition, the antibodies produced during the secondary response

are more effective and bind with higher affinity to the targeted epitopes. Plasma cells produced during secondary responses live longer than those produced during the primary response, so levels of specific antibody remain elevated for a longer period of time.



Compared to the primary response, the secondary antibody response occurs more quickly and produces antibody levels that are higher and more sustained. The secondary response mostly involves IgG.

Note:

- What events occur during the lag period of the primary antibody response?
- Why do antibody levels remain elevated longer during the secondary antibody response?

Key Concepts and Summary

- **B lymphocytes** or **B cells** produce antibodies involved in humoral immunity. B cells are produced in the bone marrow, where the initial stages of maturation occur, and travel to the spleen for final steps of maturation into naïve mature B cells.
- **B-cell receptors (BCRs)** are membrane-bound monomeric forms of IgD and IgM that bind specific antigen epitopes with their Fab antigen-binding regions. Diversity of antigen binding specificity is created by genetic rearrangement of V, D, and J segments similar to the mechanism used for TCR diversity.
- Protein antigens are called **T-dependent antigens** because they can only activate B cells with the cooperation of helper T cells. Other molecule classes do not require T cell cooperation and are called **T-independent antigens**.
- **T cell-independent activation** of B cells involves cross-linkage of BCRs by repetitive nonprotein antigen epitopes. It is characterized by the production of IgM by **plasma cells** and does not produce memory B cells.
- **T cell-dependent activation** of B cells involves processing and presentation of protein antigens to helper T cells, activation of the B cells by cytokines secreted from activated $T_{H}2$ cells, and plasma cells that produce different classes of antibodies as a result of **class switching**. **Memory B cells** are also produced.
- Secondary exposures to T-dependent antigens result in a secondary antibody response initiated by memory B cells. The secondary response develops more quickly and produces higher and more sustained levels of antibody with higher affinity for the specific antigen.

Multiple Choice

Exercise:

Problem: Which of the following would be a T-dependent antigen?

- lipopolysaccharide
- glycolipid
- protein

d. carbohydrate

Solution:

C

Exercise:

Problem: Which of the following would be a BCR?

- a. CD4
 - b. MHC II
 - c. MHC I
 - d. IgD
-

Solution:

D

Exercise:

Problem:

Which of the following does not occur during the lag period of the primary antibody response?

- a. activation of helper T cells
 - b. class switching to IgG
 - c. presentation of antigen with MHC II
 - d. binding of antigen to BCRs
-

Solution:

B

Fill in the Blank

Exercise:

Problem:

_____ antigens can stimulate B cells to become activated but require cytokine assistance delivered by helper T cells.

Solution:

T-dependent

Exercise:

Problem:

T-independent antigens can stimulate B cells to become activated and secrete antibodies without assistance from helper T cells. These antigens possess _____ antigenic epitopes that cross-link BCRs.

Solution:

repetitive

Critical Thinking

Exercise:

Problem:

A patient lacks the ability to make functioning T cells because of a genetic disorder. Would this patient's B cells be able to produce antibodies in response to an infection? Explain your answer.

LEARNING OBJECTIVES

- Compare the various kinds of artificial immunity
- Differentiate between variolation and vaccination
- Describe different types of vaccines and explain their respective advantages and disadvantages

For many diseases, prevention is the best form of treatment, and few strategies for disease prevention are as effective as vaccination. Vaccination is a form of artificial immunity. By artificially stimulating the adaptive immune defenses, a vaccine triggers memory cell production similar to that which would occur during a primary response. In so doing, the patient is able to mount a strong secondary response upon exposure to the pathogen—but without having to first suffer through an initial infection. In this section, we will explore several different kinds of artificial immunity along with various types of vaccines and the mechanisms by which they induce artificial immunity.

Classifications of Adaptive Immunity

All forms of adaptive immunity can be described as either active or passive. **Active immunity** refers to the activation of an individual's own adaptive immune defenses, whereas **passive immunity** refers to the transfer of adaptive immune defenses from another individual or animal. Active and passive immunity can be further subdivided based on whether the protection is acquired naturally or artificially.

Natural active immunity is adaptive immunity that develops after natural exposure to a pathogen ([\[link\]](#)). Examples would include the lifelong immunity that develops after recovery from a chickenpox or measles infection (although an acute infection is not always necessary to activate adaptive immunity). The length of time that an individual is protected can vary substantially depending upon the pathogen and antigens involved. For example, activation of adaptive immunity by protein spike structures during an intracellular viral infection can activate lifelong immunity, whereas activation by carbohydrate capsule antigens during an extracellular bacterial infection may activate shorter-term immunity.

Natural passive immunity involves the natural passage of antibodies from a mother to her child before and after birth. IgG is the only antibody class that can cross the placenta from mother's blood to the fetal blood supply. Placental transfer of IgG is an important passive immune defense for the infant, lasting up to six months after birth. Secretory IgA can also be transferred from mother to infant through breast milk.

Artificial passive immunity refers to the transfer of antibodies produced by a donor (human or animal) to another individual. This transfer of antibodies may be done as a prophylactic measure (i.e., to prevent disease after exposure to a pathogen) or as a strategy for treating an active infection. For

example, artificial passive immunity is commonly used for post-exposure prophylaxis against rabies, hepatitis A, hepatitis B, and chickenpox (in high risk individuals). Active infections treated by artificial passive immunity include cytomegalovirus infections in immunocompromised patients and Ebola virus infections. In 1995, eight patients in the Democratic Republic of the Congo with active Ebola infections were treated with blood transfusions from patients who were recovering from Ebola. Only one of the eight patients died (a 12.5% mortality rate), which was much lower than the expected 80% mortality rate for Ebola in untreated patients.[\[footnote\]](#) Artificial passive immunity is also used for the treatment of diseases caused by bacterial toxins, including tetanus, botulism, and diphtheria.

K. Mupapa, M. Massamba, K. Kibadi, K. Kivula, A. Bwaka, M. Kipasa, R. Colebunders, J. J. Muyembe-Tamfum. “Treatment of Ebola Hemorrhagic Fever with Blood Transfusions from Convalescent Patients.” *Journal of Infectious Diseases* 179 Suppl. (1999): S18–S23.

Artificial active immunity is the foundation for vaccination. It involves the activation of adaptive immunity through the deliberate exposure of an individual to weakened or inactivated pathogens, or preparations consisting of key pathogen antigens.

Mechanisms of Acquisition of Immunity		
	Natural acquired	Artificial acquired
Passive	Immunity acquired from antibodies passed in breast milk or through placenta 	Immunity gained through antibodies harvested from another person or an animal 
Active	Immunity gained through illness and recovery 	Immunity acquired through a vaccine 

The four classifications of immunity. (credit top left photo: modification of work by USDA; credit top right photo: modification of work by "Michaelberry"/Wikimedia; credit bottom left photo: modification of work by Centers for Disease Control and Prevention; credit bottom right photo: modification of work by Friskila Silitonga, Indonesia, Centers for Disease Control and Prevention)

Note:

- What is the difference between active and passive immunity?
- What kind of immunity is conferred by a vaccine?

Herd Immunity

The four kinds of immunity just described result from an individual's adaptive immune system. For any given disease, an individual may be considered immune or susceptible depending on his or her ability to mount an effective immune response upon exposure. Thus, any given population is likely to have some individuals who are immune and other individuals who are susceptible. If a population has very few susceptible individuals, even those susceptible individuals will be protected by a phenomenon called **herd immunity**. Herd immunity has nothing to do with an individual's ability to mount an effective immune response; rather, it occurs because there are too few susceptible individuals in a population for the disease to spread effectively.

Vaccination programs create herd immunity by greatly reducing the number of susceptible individuals in a population. Even if some individuals in the population are not vaccinated, as long as a certain percentage is immune (either naturally or artificially), the few susceptible individuals are unlikely to be exposed to the pathogen. However, because new individuals are constantly entering populations (for example, through birth or relocation), vaccination programs are necessary to maintain herd immunity.

Note:

Vaccination: Obligation or Choice

A growing number of parents are choosing not to vaccinate their children. They are dubbed "antivaxxers," and the majority of them believe that vaccines are a cause of autism (or other disease conditions), a link that has now been thoroughly disproven. Others object to vaccines on religious or moral grounds (e.g., the argument that Gardasil vaccination against HPV may promote sexual promiscuity), on personal ethical grounds (e.g., a conscientious objection to any medical intervention), or on political grounds (e.g., the notion that mandatory vaccinations are a violation of individual liberties).[\[footnote\]](#)

Elizabeth Yale. "Why Anti-Vaccination Movements Can Never Be Tamed." *Religion & Politics*, July 22, 2014. <http://religionandpolitics.org/2014/07/22/why-anti-vaccination-movements-can-never-be-tamed>.

It is believed that this growing number of unvaccinated individuals has led to new outbreaks of whooping cough and measles. We would expect that herd immunity would protect those unvaccinated in our population, but herd immunity can only be maintained if enough individuals are being vaccinated.

Vaccination is clearly beneficial for public health. But from the individual parent's perspective the view can be murkier. Vaccines, like all medical interventions, have associated risks, and while the risks of vaccination may be extremely low compared to the risks of infection, parents may not always understand or accept the consensus of the medical community. Do such parents have a right to withhold vaccination from their children? Should they be allowed to put their children—and society at large—at risk?

Many governments insist on childhood vaccinations as a condition for entering public school, but it has become easy in most states to opt out of the requirement or to keep children out of the public system. Since the 1970s, West Virginia and Mississippi have had in place a stringent requirement for childhood vaccination, without exceptions, and neither state has had a case of measles since the early 1990s. California lawmakers recently passed a similar law in response to a measles outbreak in 2015, making it much more difficult for parents to opt out of vaccines if their children are attending public schools. Given this track record and renewed legislative efforts, should other states adopt similarly strict requirements?

What role should health-care providers play in promoting or enforcing universal vaccination? Studies have shown that many parents' minds can be changed in response to information delivered by health-

care workers, but is it the place of health-care workers to try to persuade parents to have their children vaccinated? Some health-care providers are understandably reluctant to treat unvaccinated patients. Do they have the right to refuse service to patients who decline vaccines? Do insurance companies have the right to deny coverage to antivaxxers? These are all ethical questions that policymakers may be forced to address as more parents skirt vaccination norms.

Variolation and Vaccination

Thousands of years ago, it was first recognized that individuals who survived a smallpox infection were immune to subsequent infections. The practice of inoculating individuals to actively protect them from smallpox appears to have originated in the 10th century in China, when the practice of **variolation** was described ([\(link\)](#)). Variolation refers to the deliberate inoculation of individuals with infectious material from scabs or pustules of smallpox victims. Infectious materials were either injected into the skin or introduced through the nasal route. The infection that developed was usually milder than naturally acquired smallpox, and recovery from the milder infection provided protection against the more serious disease.

Although the majority of individuals treated by variolation developed only mild infections, the practice was not without risks. More serious and sometimes fatal infections did occur, and because smallpox was contagious, infections resulting from variolation could lead to epidemics. Even so, the practice of variolation for smallpox prevention spread to other regions, including India, Africa, and Europe.



Variolation for smallpox originated in the Far East and the practice later spread to Europe and Africa. This Japanese

practice later spread to Europe and Africa. This Japanese relief depicts a patient receiving a smallpox variolation from the physician Ogata Shunsaku (1748–1810).

Although variolation had been practiced for centuries, the English physician Edward Jenner (1749–1823) is generally credited with developing the modern process of vaccination. Jenner observed that milkmaids who developed cowpox, a disease similar to smallpox but milder, were immune to the more serious smallpox. This led Jenner to hypothesize that exposure to a less virulent pathogen could provide immune protection against a more virulent pathogen, providing a safer alternative to variolation. In 1796, Jenner tested his hypothesis by obtaining infectious samples from a milkmaid's active cowpox lesion and injecting the materials into a young boy ([\[link\]](#)). The boy developed a mild infection that included a low-grade fever, discomfort in his axillae (armpit) and loss of appetite. When the boy was later infected with infectious samples from smallpox lesions, he did not contract smallpox. [\[footnote\]](#) This new approach was termed **vaccination**, a name deriving from the use of cowpox (Latin *vacca* meaning “cow”) to protect against smallpox. Today, we know that Jenner’s vaccine worked because the cowpox virus is genetically and antigenically related to the *Variola* viruses that caused smallpox. Exposure to cowpox antigens resulted in a primary response and the production of memory cells that identical or related epitopes of *Variola* virus upon a later exposure to smallpox.

N. J. Willis. “Edward Jenner and the Eradication of Smallpox.” *Scottish Medical Journal* 42 (1997): 118–121.

The success of Jenner’s smallpox vaccination led other scientists to develop vaccines for other diseases. Perhaps the most notable was Louis Pasteur, who developed vaccines for rabies, cholera, and anthrax. During the 20th and 21st centuries, effective vaccines were developed to prevent a wide range of diseases caused by viruses (e.g., chickenpox and shingles, hepatitis, measles, mumps, polio, and yellow fever) and bacteria (e.g., diphtheria, pneumococcal pneumonia, tetanus, and whooping cough.).



(a)



(b)

(a) A painting of Edward Jenner depicts a cow and a milkmaid in the background. (b) Lesions on a patient infected with cowpox, a zoonotic disease caused by a virus closely related to the one that

causes smallpox. (credit b: modification of work by the Centers for Disease Control and Prevention)

Note:

- What is the difference between variolation and vaccination for smallpox?
- Explain why vaccination is less risky than variolation.

Classes of Vaccines

For a vaccine to provide protection against a disease, it must expose an individual to pathogen-specific antigens that will stimulate a protective adaptive immune response. By its very nature, this entails some risk. As with any pharmaceutical drug, vaccines have the potential to cause adverse effects. However, the ideal vaccine causes no severe adverse effects and poses no risk of contracting the disease that it is intended to prevent. Various types of vaccines have been developed with these goals in mind. These different classes of vaccines are described in the next section and summarized in [\[link\]](#).

Live Attenuated Vaccines

Live attenuated vaccines expose an individual to a weakened strain of a pathogen with the goal of establishing a subclinical infection that will activate the adaptive immune defenses. Pathogens are attenuated to decrease their virulence using methods such as genetic manipulation (to eliminate key virulence factors) or long-term culturing in an unnatural host or environment (to promote mutations and decrease virulence).

By establishing an active infection, live attenuated vaccines stimulate a more comprehensive immune response than some other types of vaccines. Live attenuated vaccines activate both cellular and humoral immunity and stimulate the development of memory for long-lasting immunity. In some cases, vaccination of one individual with a live attenuated pathogen can even lead to natural transmission of the attenuated pathogen to other individuals. This can cause the other individuals to also develop an active, subclinical infection that activates their adaptive immune defenses.

Disadvantages associated with live attenuated vaccines include the challenges associated with long-term storage and transport as well as the potential for a patient to develop signs and symptoms of disease during the active infection (particularly in immunocompromised patients). There is also a risk of the attenuated pathogen reverting back to full virulence. [\[link\]](#) lists examples live attenuated vaccines.

Inactivated Vaccines

Inactivated vaccines contain whole pathogens that have been killed or inactivated with heat, chemicals, or radiation. For inactivated vaccines to be effective, the inactivation process must not affect the structure of key antigens on the pathogen.

Because the pathogen is killed or inactive, inactivated vaccines do not produce an active infection, and the resulting immune response is weaker and less comprehensive than that provoked by a live attenuated vaccine. Typically the response involves only humoral immunity, and the pathogen cannot be transmitted to other individuals. In addition, inactivated vaccines usually require higher doses and multiple boosters, possibly causing inflammatory reactions at the site of injection.

Despite these disadvantages, inactivated vaccines do have the advantages of long-term storage stability and ease of transport. Also, there is no risk of causing severe active infections. However, inactivated vaccines are not without their side effects. [\[link\]](#) lists examples of inactivated vaccines.

Subunit Vaccines

Whereas live attenuated and inactive vaccines expose an individual to a weakened or dead pathogen, **subunit vaccines** only expose the patient to the key antigens of a pathogen—not whole cells or viruses. Subunit vaccines can be produced either by chemically degrading a pathogen and isolating its key antigens or by producing the antigens through genetic engineering. Because these vaccines contain only the essential antigens of a pathogen, the risk of side effects is relatively low. [\[link\]](#) lists examples of subunit vaccines.

Toxoid Vaccines

Like subunit vaccines, **toxoid vaccines** do not introduce a whole pathogen to the patient; they contain inactivated bacterial toxins, called toxoids. Toxoid vaccines are used to prevent diseases in which bacterial toxins play an important role in pathogenesis. These vaccines activate humoral immunity that neutralizes the toxins. [\[link\]](#) lists examples of toxoid vaccines.

Conjugate Vaccines

A **conjugate vaccine** is a type of subunit vaccine that consists of a protein conjugated to a capsule polysaccharide. Conjugate vaccines have been developed to enhance the efficacy of subunit vaccines against pathogens that have protective polysaccharide capsules that help them evade phagocytosis, causing invasive infections that can lead to meningitis and other serious conditions. The subunit vaccines against these pathogens introduce T-independent capsular polysaccharide antigens that result in the production of antibodies that can opsonize the capsule and thus combat the infection; however, children under the age of two years do not respond effectively to these vaccines. Children do respond effectively when vaccinated with the conjugate vaccine, in which a protein with T-dependent antigens is conjugated to the capsule polysaccharide. The conjugated protein-polysaccharide antigen stimulates production of antibodies against both the protein and the capsule polysaccharide. [\[link\]](#) lists examples of conjugate vaccines.

Classes of Vaccines				
Class	Description	Advantages	Disadvantages	Examples
Live attenuated	Weakened strain of whole pathogen	Cellular and humoral immunity	Difficult to store and transport	Chickenpox, German measles, measles, mumps, tuberculosis, typhoid fever, yellow fever
		Long-lasting immunity	Risk of infection in immunocompromised patients	
		Transmission to contacts	Risk of reversion	
Inactivated	Whole pathogen killed or inactivated with heat, chemicals, or radiation	Ease of storage and transport	Weaker immunity (humoral only)	Cholera, hepatitis A, influenza, plague, rabies
		No risk of severe active infection	Higher doses and more boosters required	
Subunit	Immunogenic antigens	Lower risk of side effects	Limited longevity	Anthrax, hepatitis B, influenza, meningitis, papillomavirus, pneumococcal pneumonia, whooping cough
			Multiple doses required	
			No protection against antigenic variation	
Toxoid	Inactivated bacterial toxin	Humoral immunity to neutralize toxin	Does not prevent infection	Botulism, diphtheria, pertussis, tetanus
Conjugate	Capsule polysaccharide conjugated to protein	T-dependent response to capsule	Costly to produce	Meningitis (<i>Haemophilus influenzae</i> , <i>Streptococcus pneumoniae</i> , <i>Neisseria meningitidis</i>)
			No protection against antigenic variation	
		Better response in young children	May interfere with other vaccines	

Note:

- What is the risk associated with a live attenuated vaccine?
- Why is a conjugated vaccine necessary in some cases?

Note:**DNA Vaccines**

DNA vaccines represent a relatively new and promising approach to vaccination. A DNA vaccine is produced by incorporating genes for antigens into a recombinant plasmid vaccine. Introduction of the DNA vaccine into a patient leads to uptake of the recombinant plasmid by some of the patient's cells, followed by transcription and translation of antigens and presentation of these antigens with MHC I to activate adaptive immunity. This results in the stimulation of both humoral and cellular immunity without the risk of active disease associated with live attenuated vaccines.

Although most DNA vaccines for humans are still in development, it is likely that they will become more prevalent in the near future as researchers are working on engineering DNA vaccines that will activate adaptive immunity against several different pathogens at once. First-generation DNA vaccines tested in the 1990s looked promising in animal models but were disappointing when tested in human subjects. Poor cellular uptake of the DNA plasmids was one of the major problems impacting their efficacy. Trials of second-generation DNA vaccines have been more promising thanks to new techniques for enhancing cellular uptake and optimizing antigens. DNA vaccines for various cancers and viral pathogens such as HIV, HPV, and hepatitis B and C are currently in development.

Some DNA vaccines are already in use. In 2005, a DNA vaccine against West Nile virus was approved for use in horses in the United States. Canada has also approved a DNA vaccine to protect fish from infectious hematopoietic necrosis virus.[\[footnote\]](#) A DNA vaccine against Japanese encephalitis virus was approved for use in humans in 2010 in Australia.[\[footnote\]](#)

M. Alonso and J. C. Leong. "Licensed DNA Vaccines Against Infectious Hematopoietic Necrosis Virus (IHNV)." *Recent Patents on DNA & Gene Sequences (Discontinued)* 7 no. 1 (2013): 62–65, issn 1872-2156/2212-3431. doi 10.2174/1872215611307010009.

S.B. Halstead and S. J. Thomas. "New Japanese Encephalitis Vaccines: Alternatives to Production in Mouse Brain." *Expert Review of Vaccines* 10 no. 3 (2011): 355–64.

Note:**Resolution**

Based on Olivia's symptoms, her physician made a preliminary diagnosis of bacterial meningitis without waiting for positive identification from the blood and CSF samples sent to the lab. Olivia was admitted to the hospital and treated with intravenous broad-spectrum antibiotics and rehydration therapy. Over the next several days, her condition began to improve, and new blood samples and lumbar puncture samples showed an absence of microbes in the blood and CSF with levels of white blood cells returning to normal. During this time, the lab produced a positive identification of *Neisseria meningitidis*, the causative agent of meningococcal meningitis, in her original CSF sample. *N. meningitidis* produces a polysaccharide capsule that serves as a virulence factor. *N. meningitidis* tends to affect infants after they begin to lose the natural passive immunity provided by maternal antibodies. At one year of age, Olivia's maternal IgG antibodies would have disappeared, and she would not have developed memory cells capable of recognizing antigens associated with the polysaccharide capsule of the *N. meningitidis*. As a result, her adaptive immune system was unable to produce protective antibodies to combat the infection, and without antibiotics she may not have

survived. Olivia's infection likely would have been avoided altogether had she been vaccinated. A conjugate vaccine to prevent meningococcal meningitis is available and approved for infants as young as two months of age. However, current vaccination schedules in the United States recommend that the vaccine be administered at age 11–12 with a booster at age 16.

Go back to the [previous](#) Clinical Focus box.

Note:



In countries with developed public health systems, many vaccines are routinely administered to children and adults. Vaccine schedules are changed periodically, based on new information and research results gathered by public health agencies. In the United States, the CDC publishes [schedules and other updated information](#) about vaccines.

Key Concepts and Summary

- Adaptive immunity can be divided into four distinct classifications: **natural active immunity**, **natural passive immunity**, **artificial passive immunity**, and **artificial active immunity**.
- Artificial active immunity is the foundation for **vaccination** and vaccine development. Vaccination programs not only confer artificial immunity on individuals, but also foster **herd immunity** in populations.
- **Variolation** against smallpox originated in the 10th century in China, but the procedure was risky because it could cause the disease it was intended to prevent. Modern vaccination was developed by Edward Jenner, who developed the practice of inoculating patients with infectious materials from cowpox lesions to prevent smallpox.
- **Live attenuated vaccines** and **inactivated vaccines** contain whole pathogens that are weak, killed, or inactivated. **Subunit vaccines**, **toxoid vaccines**, and **conjugate vaccines** contain acellular components with antigens that stimulate an immune response.

Multiple Choice

Exercise:

Problem:

A patient is bitten by a dog with confirmed rabies infection. After treating the bite wound, the physician injects the patient with antibodies that are specific for the rabies virus to prevent the development of an active infection. This is an example of:

- a. Natural active immunity

- b. Artificial active immunity
 - c. Natural passive immunity
 - d. Artificial passive immunity
-

Solution:

D

Exercise:

Problem:

A patient gets a cold, and recovers a few days later. The patient's classmates come down with the same cold roughly a week later, but the original patient does not get the same cold again. This is an example of:

- a. Natural active immunity
 - b. Artificial active immunity
 - c. Natural passive immunity
 - d. Artificial passive immunity
-

Solution:

A

Matching

Exercise:

Problem: Match each type of vaccine with the corresponding example.

___ inactivated vaccine	A. Weakened influenza virions that can only replicate in the slightly lower temperatures of the nasal passages are sprayed into the nose. They do not cause serious flu symptoms, but still produce an active infection that induces a protective adaptive immune response.
___ live attenuated vaccine	B. Tetanus toxin molecules are harvested and chemically treated to render them harmless. They are then injected into a patient's arm.
___ toxoid vaccine	C. Influenza virus particles grown in chicken eggs are harvested and chemically treated to render them

noninfectious. These immunogenic particles are then purified and packaged and administered as an injection.

subunit vaccine

D. The gene for hepatitis B virus surface antigen is inserted into a yeast genome. The modified yeast is grown and the virus protein is produced, harvested, purified, and used in a vaccine.

Solution:

C, A, B, D

Fill in the Blank

Exercise:

Problem:

A(n) _____ pathogen is in a weakened state; it is still capable of stimulating an immune response but does not cause a disease.

Solution:

attenuated

Exercise:

Problem:

_____ immunity occurs when antibodies from one individual are harvested and given to another to protect against disease or treat active disease.

Solution:

Artificial passive

Exercise:

Problem:

In the practice of _____, scabs from smallpox victims were used to immunize susceptible individuals against smallpox.

Solution:

variolation

Short answer

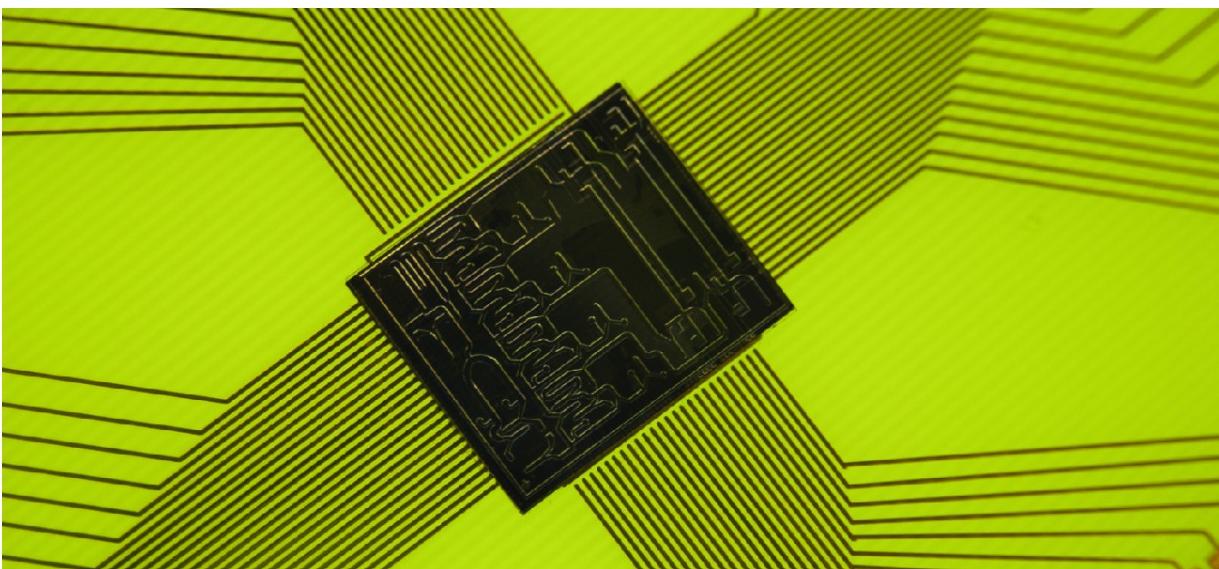
Exercise:

Problem: Briefly compare the pros and cons of inactivated versus live attenuated vaccines.

Introduction class="introduction"

Lab-on-a-chip technology allows immunological assays to be miniaturized so tests can be done rapidly with minimum quantities of expensive reagents. The chips contain tiny flow tubes to allow movement of fluids by capillary action, reactions sites with embedded reagents, and data output through electronic sensors.

(credit:
modification
of work by
Maggie
Bartlett,
NHGRI)



Many laboratory tests are designed to confirm a presumptive diagnosis by detecting antibodies specific to a suspected pathogen. Unfortunately, many such tests are time-consuming and expensive. That is now changing, however, with the development of new, miniaturized technologies that are fast and inexpensive. For example, researchers at Columbia University are developing a “lab-on-a-chip” technology that will test a single drop of blood for 15 different infectious diseases, including HIV and syphilis, in a matter of minutes.[\[footnote\]](#) The blood is pulled through tiny capillaries into reaction chambers where the patient’s antibodies mix with reagents. A chip reader that attaches to a cell phone analyzes the results and sends them to the patient’s healthcare provider. Currently the device is being field tested in Rwanda to check pregnant women for chronic diseases. Researchers estimate that the chip readers will sell for about \$100 and individual chips for \$1.[\[footnote\]](#)

Chin, Curtis D. et al., “Mobile Device for Disease Diagnosis and Data Tracking in Resource-Limited Settings,” *Clinical Chemistry* 59, no. 4 (2013): 629-40.

Evarts, H., “Fast, Low-Cost Device Uses the Cloud to Speed Up Testing for HIV and More,” January 24, 2013. Accessed July 14, 2016.

<http://engineering.columbia.edu/fast-low-cost-device-uses-cloud-speed-diagnostic-testing-hiv-and-more>.

Polyclonal and Monoclonal Antibody Production

LEARNING OBJECTIVES

- Compare the method of development, use, and characteristics of monoclonal and polyclonal antibodies
- Explain the nature of antibody cross-reactivity and why this is less of a problem with monoclonal antibodies

Note:

Part 1

In an unfortunate incident, a healthcare worker struggling with addiction was caught stealing syringes of painkillers and replacing them with syringes filled with unknown substances. The hospital immediately fired the employee and had him arrested; however, two patients that he had worked with later tested positive for HIV.

While there was no proof that the infections originated from the tainted syringes, the hospital's public health physician took immediate steps to determine whether any other patients had been put at risk. Although the worker had only been employed for a short time, it was determined that he had come into contact with more than 1300 patients. The hospital decided to contact all of these patients and have them tested for HIV.

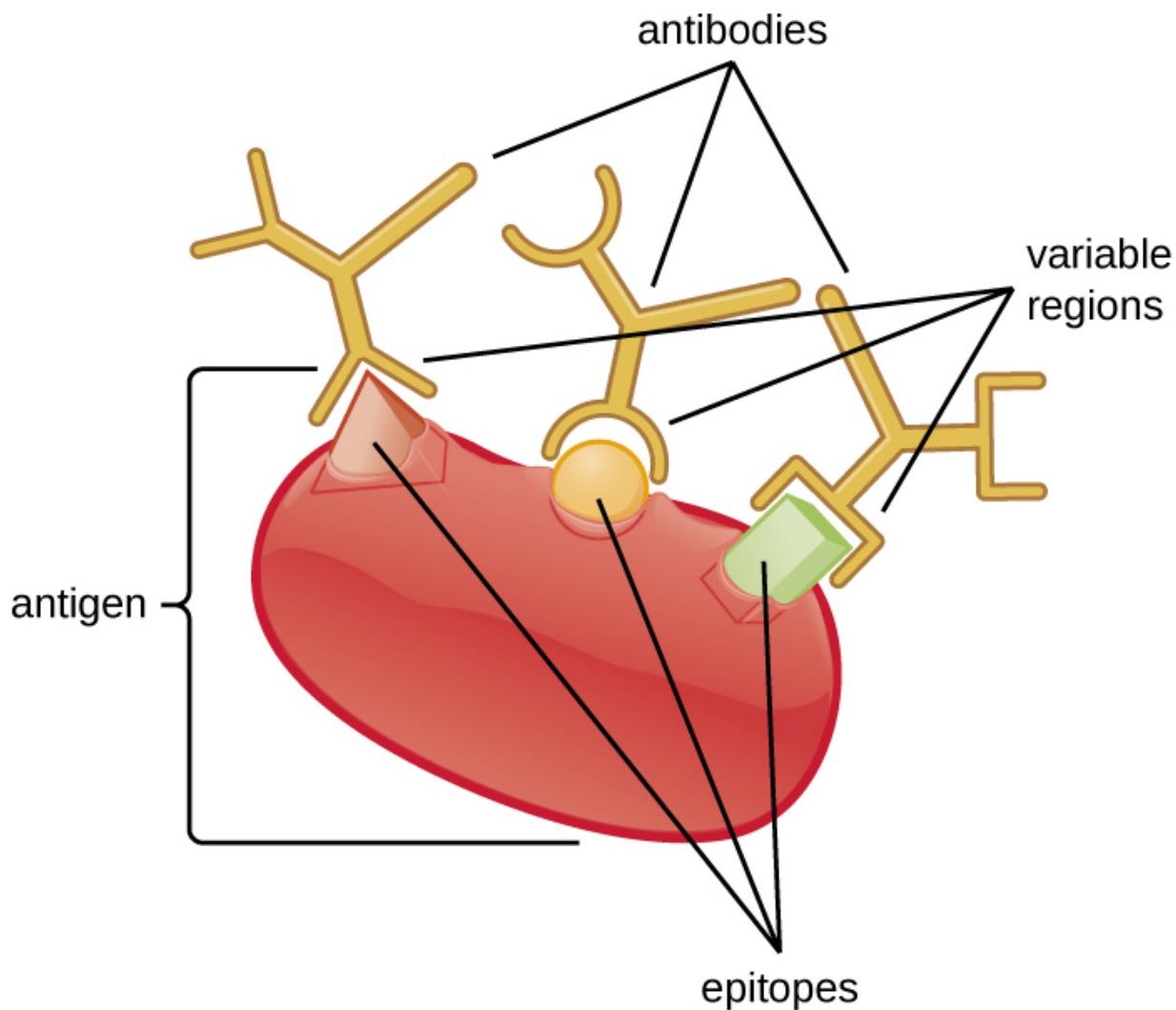
- Why does the hospital feel it is necessary to test every patient for HIV?
- What types of tests can be used to determine if a patient has HIV?

Jump to the [next](#) Clinical Focus box.

In addition to being crucial for our normal immune response, antibodies provide powerful tools for research and diagnostic purposes. The high specificity of antibodies makes them an excellent tool for detecting and quantifying a broad array of targets, from drugs to serum proteins to microorganisms. With *in vitro* assays, antibodies can be used to precipitate soluble antigens, agglutinate (clump) cells, opsonize and kill bacteria with the assistance of complement, and neutralize drugs, toxins, and viruses.

An antibody's **specificity** results from the antigen-binding site formed within the variable regions—regions of the antibody that have unique patterns of amino acids that can only bind to target antigens with a molecular sequence that provides complementary charges and noncovalent bonds. There are limitations to antibody specificity, however. Some antigens are so chemically similar that cross-reactivity occurs; in other words, antibodies raised against one antigen bind to a chemically similar but different antigen. Consider an antigen that consists of a single protein with multiple epitopes ([\[link\]](#)). This single protein may stimulate the production of many different antibodies, some of which may bind to chemically identical epitopes on other proteins.

Cross-reactivity is more likely to occur between antibodies and antigens that have low **affinity** or **avidity**. Affinity, which can be determined experimentally, is a measure of the binding strength between an antibody's binding site and an epitope, whereas avidity is the total strength of all the interactions in an antibody-antigen complex (which may have more than one bonding site). Avidity is influenced by affinity as well as the structural arrangements of the epitope and the variable regions of the antibody. If an antibody has a high affinity/avidity for a specific antigen, it is less likely to cross-react with an antigen for which it has a lower affinity/avidity.



An antibody binds to a specific region on an antigen called an epitope.

A single antigen can have multiple epitopes for different, specific antibodies.

Note:

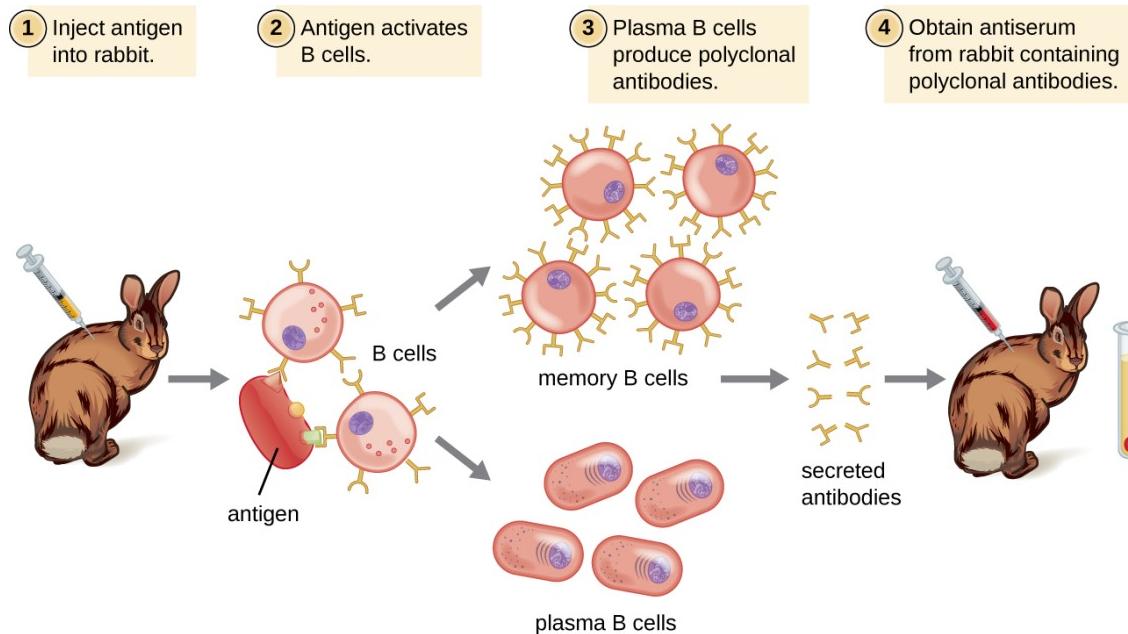
- What property makes antibodies useful for research and clinical diagnosis?
- What is cross-reactivity and why does it occur?

Producing Polyclonal Antibodies

Antibodies used for research and diagnostic purposes are often obtained by injecting a lab animal such as a rabbit or a goat with a specific antigen. Within a few weeks, the animal's immune system will produce high levels of antibodies specific for the antigen. These antibodies can be harvested in an **antiserum**, which is whole serum collected from an animal following exposure to an antigen. Because most antigens are complex structures with multiple epitopes, they result in the production of multiple antibodies in the lab animal. This so-called **polyclonal antibody** response is also typical of the response to infection by the human immune system. Antiserum drawn from an animal will thus contain antibodies from multiple clones of B cells, with each B cell responding to a specific epitope on the antigen ([\[link\]](#)).

Lab animals are usually injected at least twice with antigen when being used to produce antiserum. The second injection will activate memory cells that make class IgG antibodies against the antigen. The memory cells also undergo **affinity maturation**, resulting in a pool of antibodies with higher average affinity. Affinity maturation occurs because of mutations in the immunoglobulin gene variable regions, resulting in B cells with slightly altered antigen-binding sites. On re-exposure to the antigen, those B cells capable of producing antibody with higher affinity antigen-binding sites will be stimulated to proliferate and produce more antibody than their lower-affinity peers. An adjuvant, which is a chemical that provokes a generalized activation of the immune system that stimulates greater antibody production, is often mixed with the antigen prior to injection.

Antiserum obtained from animals will not only contain antibodies against the antigen artificially introduced in the laboratory, but it will also contain antibodies to any other antigens to which the animal has been exposed during its lifetime. For this reason, antisera must first be “purified” to remove other antibodies before using the antibodies for research or diagnostic assays.



This diagram illustrates the process for harvesting polyclonal antibodies produced in response to an antigen.

Clinical Uses of Polyclonal Antisera

Polyclonal antisera are used in many clinical tests that are designed to determine whether a patient is producing antibodies in response to a particular pathogen. While these tests are certainly powerful diagnostic tools, they have their limitations, because they are an indirect means of determining whether a particular pathogen is present. Tests based on a polyclonal response can sometimes lead to a **false-positive** result—in other words, a test that confirms the presence of an antigen that is, in fact, not present. Antibody-based tests can also result in a **false-negative** result, which occurs when the test fails to detect an antibody that is, in fact, present.

The accuracy of antibody tests can be described in terms of **test sensitivity** and **test specificity**. Test sensitivity is the probability of getting a positive test result when the patient is indeed infected. If a test has high sensitivity, the probability of a false negative is low. Test specificity, on the other hand,

is the probability of getting a negative test result when the patient is not infected. If a test has high specificity, the probability of a false positive is low.

False positives often occur due to cross-reactivity, which can occur when epitopes from a different pathogen are similar to those found on the pathogen being tested for. For this reason, antibody-based tests are often used only as screening tests; if the results are positive, other confirmatory tests are used to make sure that the results were not a false positive.

For example, a blood sample from a patient suspected of having hepatitis C can be screened for the virus using antibodies that bind to antigens on hepatitis C virus. If the patient is indeed infected with hepatitis C virus, the antibodies will bind to the antigens, yielding a positive test result. If the patient is not infected with hepatitis C virus, the antibodies will generally not bind to anything and the test should be negative; however, a false positive may occur if the patient has been previously infected by any of a variety of pathogens that elicit antibodies that cross-react with the hepatitis C virus antigens. Antibody tests for hepatitis C have high sensitivity (a low probability of a false negative) but low specificity (a high probability of a false positive). Thus, patients who test positive must have a second, confirmatory test to rule out the possibility of a false positive. The confirmatory test is a more expensive and time-consuming test that directly tests for the presence of hepatitis C viral RNA in the blood. Only after the confirmatory test comes back positive can the patient be definitively diagnosed with a hepatitis C infection. Antibody-based tests can result in a false negative if, for any reason, the patient's immune system has not produced detectable levels of antibodies. For some diseases, it may take several weeks following infection before the immune system produces enough antibodies to cross the detection threshold of the assay. In immunocompromised patients, the immune system may not be capable of producing a detectable level of antibodies.

Another limitation of using antibody production as an indicator of disease is that antibodies in the blood will persist long after the infection has been cleared. Depending on the type of infection, antibodies will be present for many months; sometimes, they may be present for the remainder of the

patient's life. Thus, a positive antibody-based test only means that the patient was infected at some point in time; it does not prove that the infection is active.

In addition to their role in diagnosis, polyclonal antisera can activate complement, detect the presence of bacteria in clinical and food industry settings, and perform a wide array of precipitation reactions that can detect and quantify serum proteins, viruses, or other antigens. However, with the many specificities of antibody present in a polyclonal antiserum, there is a significant likelihood that the antiserum will cross-react with antigens to which the individual was never exposed. Therefore, we must always account for the possibility of false-positive results when working with a polyclonal antiserum.

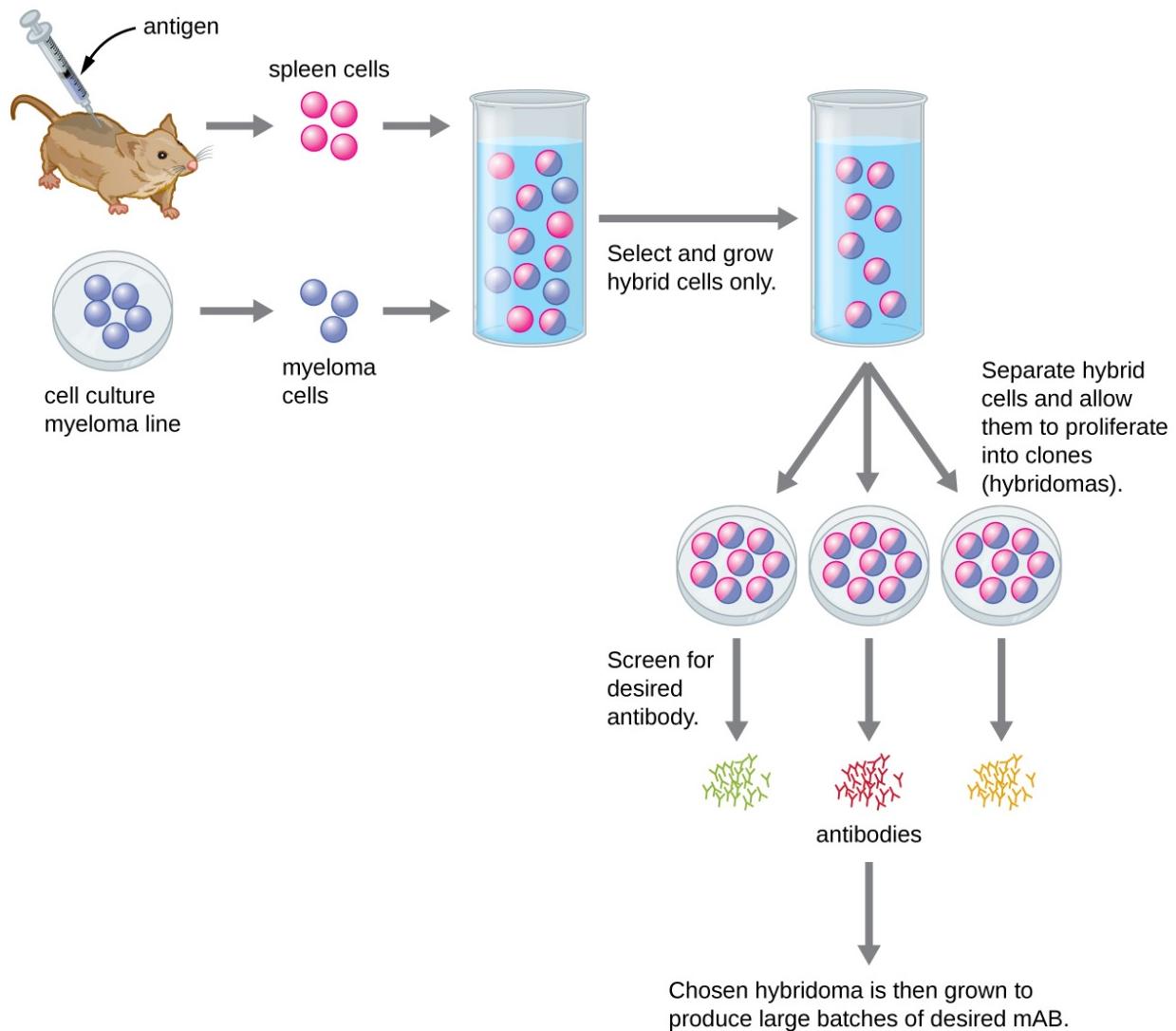
Note:

- What is a false positive and what are some reasons that false positives occur?
- What is a false negative and what are some reasons that false positives occur?
- If a patient tests negative on a highly sensitive test, what is the likelihood that the person is infected with the pathogen?

Producing Monoclonal Antibodies

Some types of assays require better antibody specificity and affinity than can be obtained using a polyclonal antiserum. To attain this high specificity, all of the antibodies must bind with high affinity to a single epitope. This high specificity can be provided by **monoclonal antibodies (mAbs)**. [\[link\]](#) compares some of the important characteristics of monoclonal and polyclonal antibodies.

Unlike polyclonal antibodies, which are produced in live animals, monoclonal antibodies are produced *in vitro* using tissue-culture techniques. mAbs are produced by immunizing an animal, often a mouse, multiple times with a specific antigen. B cells from the spleen of the immunized animal are then removed. Since normal B cells are unable to proliferate forever, they are fused with immortal, cancerous B cells called myeloma cells, to yield **hybridoma** cells. All of the cells are then placed in a selective medium that allows only the hybridomas to grow; unfused myeloma cells cannot grow, and any unfused B cells die off. The hybridomas, which are capable of growing continuously in culture while producing antibodies, are then screened for the desired mAb. Those producing the desired mAb are grown in tissue culture; the culture medium is harvested periodically and mAbs are purified from the medium. This is a very expensive and time-consuming process. It may take weeks of culturing and many liters of media to provide enough mAbs for an experiment or to treat a single patient. mAbs are expensive ([\[link\]](#)).



Monoclonal antibodies (mAbs) are produced by introducing an antigen to a mouse and then fusing polyclonal B cells from the mouse's spleen to myeloma cells. The resulting hybridoma cells are cultured and continue to produce antibodies to the antigen. Hybridomas producing the desired mAb are then grown in large numbers on a selective medium that is periodically harvested to obtain the desired mAbs.

Characteristics of Polyclonal and Monoclonal Antibodies	
Monoclonal Antibodies	Polyclonal Antibodies
Expensive production	Inexpensive production
Long production time	Rapid production
Large quantities of specific antibodies	Large quantities of nonspecific antibodies
Recognize a single epitope on an antigen	Recognize multiple epitopes on an antigen
Production is continuous and uniform once the hybridoma is made	Different batches vary in composition

Clinical Uses of Monoclonal Antibodies

Since the most common methods for producing monoclonal antibodies use mouse cells, it is necessary to create **humanized monoclonal antibodies** for human clinical use. Mouse antibodies cannot be injected repeatedly into humans, because the immune system will recognize them as being foreign and will respond to them with neutralizing antibodies. This problem can be minimized by genetically engineering the antibody in the mouse B cell. The variable regions of the mouse light and heavy chain genes are ligated to human constant regions, and the chimeric gene is then transferred into a host cell. This allows production of a mAb that is mostly “human” with only the antigen-binding site being of mouse origin.

Humanized mAbs have been successfully used to treat cancer with minimal side effects. For example, the humanized monoclonal antibody drug Herceptin has been helpful for the treatment of some types of breast cancer. There have also been a few preliminary trials of humanized mAb for the treatment of infectious diseases, but none of these treatments are currently in use. In some cases, mAbs have proven too specific to treat infectious

diseases, because they recognize some serovars of a pathogen but not others. Using a cocktail of multiple mAbs that target different strains of the pathogen can address this problem. However, the great cost associated with mAb production is another challenge that has prevented mAbs from becoming practical for use in treating microbial infections.[\[footnote\]](#) Saylor, Carolyn, Ekaterina Dadachova and Arturo Casadevall, “Monoclonal Antibody-Based Therapies for Microbial Diseases,” *Vaccine* 27 (2009): G38-G46.

One promising technology for inexpensive mAbs is the use of genetically engineered plants to produce antibodies (or **plantibodies**). This technology transforms plant cells into antibody factories rather than relying on tissue culture cells, which are expensive and technically demanding. In some cases, it may even be possible to deliver these antibodies by having patients eat the plants rather than by extracting and injecting the antibodies. For example, in 2013, a research group cloned antibody genes into plants that had the ability to neutralize an important toxin from bacteria that can cause severe gastrointestinal disease.[\[footnote\]](#) Eating the plants could potentially deliver the antibodies directly to the toxin.

Nakanishi, Katsuhiro et al., “Production of Hybrid-IgG/IgA Plantibodies with Neutralizing Activity against Shiga Toxin 1,” *PloS One* 8, no. 11 (2013): e80712.

Note:

- How are humanized monoclonal antibodies produced?
- What does the “monoclonal” of monoclonal antibodies mean?

Note:

Using Monoclonal Antibodies to Combat Ebola

During the 2014–2015 Ebola outbreak in West Africa, a few Ebola-infected patients were treated with ZMapp, a drug that had been shown to be effective in trials done in rhesus macaques only a few months before.

[footnote] ZMapp is a combination of three mAbs produced by incorporating the antibody genes into tobacco plants using a viral vector. By using three mAbs, the drug is effective across multiple strains of the virus. Unfortunately, there was only enough ZMapp to treat a tiny number of patients.

Qiu, Xiangguo et al., “Reversion of Advanced Ebola Virus Disease in Nonhuman Primates with ZMapp,” *Nature* 514 (2014): 47–53.

While the current technology is not adequate for producing large quantities of ZMapp, it does show that plantibodies—plant-produced mAbs—are feasible for clinical use, potentially cost effective, and worth further development. The last several years have seen an explosion in the number of new mAb-based drugs for the treatment of cancer and infectious diseases; however, the widespread use of such drugs is currently inhibited by their exorbitant cost, especially in underdeveloped parts of the world, where a single dose might cost more than the patient’s lifetime income. Developing methods for cloning antibody genes into plants could reduce costs dramatically.

Key Concepts and Summary

- Antibodies bind with high **specificity** to antigens used to challenge the immune system, but they may also show **cross-reactivity** by binding to other antigens that share chemical properties with the original antigen.
- Injection of an antigen into an animal will result in a **polyclonal antibody** response in which different antibodies are produced that react with the various epitopes on the antigen.
- **Polyclonal antisera** are useful for some types of laboratory assays, but other assays require more specificity. Diagnostic tests that use polyclonal antisera are typically only used for screening because of the possibility of **false-positive** and **false-negative** results.
- **Monoclonal antibodies** provide higher specificity than polyclonal antisera because they bind to a single epitope and usually have high **affinity**.

- Monoclonal antibodies are typically produced by culturing antibody-secreting **hybridomas** derived from mice. mAbs are currently used to treat cancer, but their exorbitant cost has prevented them from being used more widely to treat infectious diseases. Still, their potential for laboratory and clinical use is driving the development of new, cost-effective solutions such as **plantibodies**.

Multiple Choice

Exercise:

Problem:

For many uses in the laboratory, polyclonal antibodies work well, but for some types of assays, they lack sufficient _____ because they cross-react with inappropriate antigens.

- a. specificity
 - b. sensitivity
 - c. accuracy
 - d. reactivity
-

Solution:

A

Exercise:

Problem: How are monoclonal antibodies produced?

- a. Antibody-producing B cells from a mouse are fused with myeloma cells and then the cells are grown in tissue culture.
- b. A mouse is injected with an antigen and then antibodies are harvested from its serum.
- c. They are produced by the human immune system as a natural response to an infection.

- d. They are produced by a mouse's immune system as a natural response to an infection.
-

Solution:

A

Fill in the Blank

Exercise:

Problem:

When we inject an animal with the same antigen a second time a few weeks after the first, _____ takes place, which means the antibodies produced after the second injection will on average bind the antigen more tightly.

Solution:

affinity maturation

Exercise:

Problem:

When using mAbs to treat disease in humans, the mAbs must first be _____ by replacing the mouse constant region DNA with human constant region DNA.

Solution:

humanized

Exercise:

Problem:

If we used normal mouse mAbs to treat human disease, multiple doses would cause the patient to respond with _____ against the mouse antibodies.

Solution:

neutralizing antibodies

Exercise:**Problem:**

A polyclonal response to an infection occurs because most antigens have multiple _____,

Solution:

epitopes

Short Answer**Exercise:****Problem:**

Describe two reasons why polyclonal antibodies are more likely to exhibit cross-reactivity than monoclonal antibodies.

Critical Thinking**Exercise:**

Problem:

Suppose you were screening produce in a grocery store for the presence of *E. coli* contamination. Would it be better to use a polyclonal anti-*E. coli* antiserum or a mAb against an *E. coli* membrane protein? Explain.

Detecting Antigen-Antibody Complexes

LEARNING OBJECTIVES

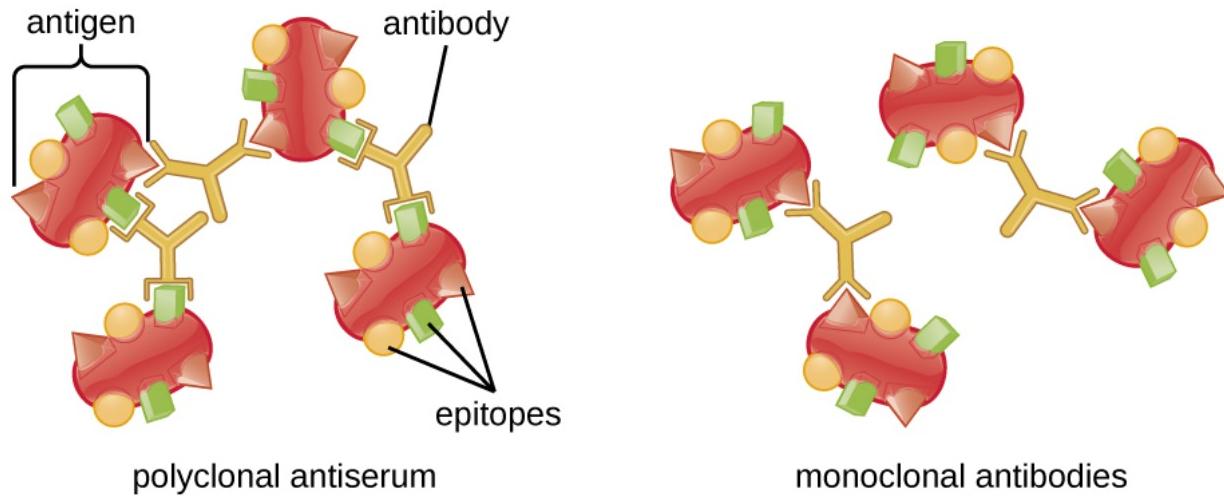
- Describe various types of assays used to find antigen-antibody complexes
- Describe the circumstances under which antigen-antibody complexes precipitate out of solution
- Explain how antibodies in patient serum can be used to diagnose disease

Laboratory tests to detect antibodies and antigens outside of the body (e.g., in a test tube) are called *in vitro* assays. When both antibodies and their corresponding antigens are present in a solution, we can often observe a precipitation reaction in which large complexes (lattices) form and settle out of solution. In the next several sections, we will discuss several common *in vitro* assays.

Precipitin Reactions

A visible antigen-antibody complex is called a **precipitin**, and *in vitro* assays that produce a precipitin are called precipitin reactions. A precipitin reaction typically involves adding soluble antigens to a test tube containing a solution of antibodies. Each antibody has two arms, each of which can bind to an epitope. When an antibody binds to two antigens, the two antigens become bound together by the antibody. A lattice can form as antibodies bind more and more antigens together, resulting in a precipitin ([\[link\]](#)). Most precipitin tests use a polyclonal antiserum rather than monoclonal antibodies because polyclonal antibodies can bind to multiple

epitopes, making lattice formation more likely. Although mAbs may bind some antigens, the binding will occur less often, making it much less likely that a visible precipitin will form.

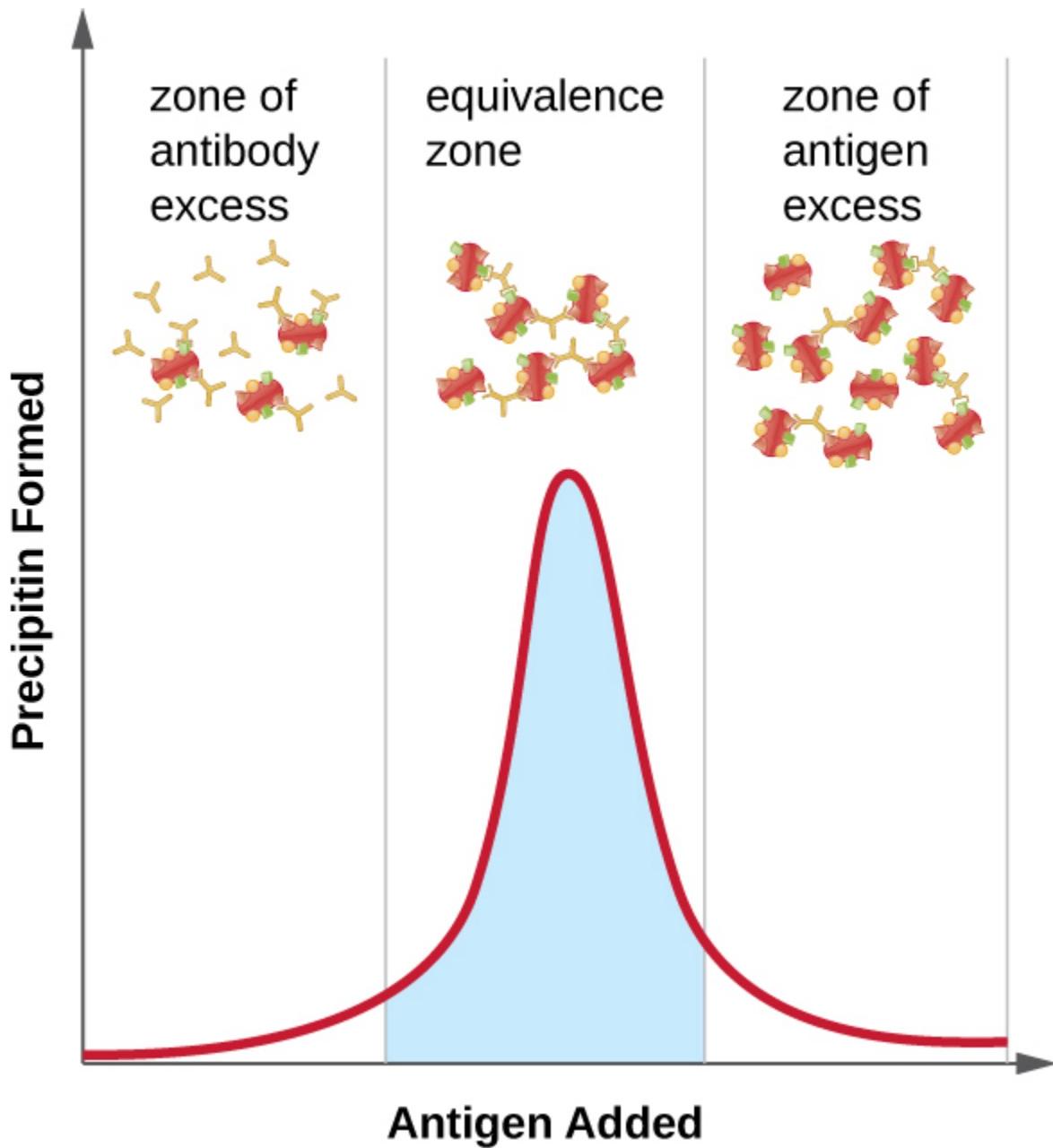


Polyclonal antiserum binds to multiple epitopes on an antigen, leading to lattice formation that results in a visible precipitin. Monoclonal antibodies can only bind to a single epitope; therefore, less binding occurs and lattice formation generally does not occur.

The amount of precipitation also depends on several other factors. For example, precipitation is enhanced when the antibodies have a high affinity for the antigen. While most antibodies bind antigen with high affinity, even high-affinity binding uses relatively weak noncovalent bonds, so that individual interactions will often break and new interactions will occur.

In addition, for precipitin formation to be visible, there must be an optimal ratio of antibody to antigen. The optimal ratio is not likely to be a 1:1 antigen-to-antibody ratio; it can vary dramatically, depending on the number of epitopes on the antigen and the class of antibody. Some antigens may have only one or two epitopes recognized by the antiserum, whereas other antigens may have many different epitopes and/or multiple instances of the same epitope on a single antigen molecule.

[\[link\]](#) illustrates how the ratio of antigen and antibody affects the amount of precipitation. To achieve the optimal ratio, antigen is slowly added to a solution containing antibodies, and the amount of precipitin is determined qualitatively. Initially, there is not enough antigen to produce visible lattice formation; this is called the zone of antibody excess. As more antigen is added, the reaction enters the **equivalence zone** (or zone of equivalence), where both the optimal antigen-antibody interaction and maximal precipitation occur. If even more antigen were added, the amount of antigen would become excessive and actually cause the amount of precipitation to decline.



As antigen is slowly added to a solution containing a constant amount antibody, the amount of precipitin increases as the antibody-to-antigen ratio approaches the equivalence zone and decreases once the proportion of antigen exceeds the optimal ratio.

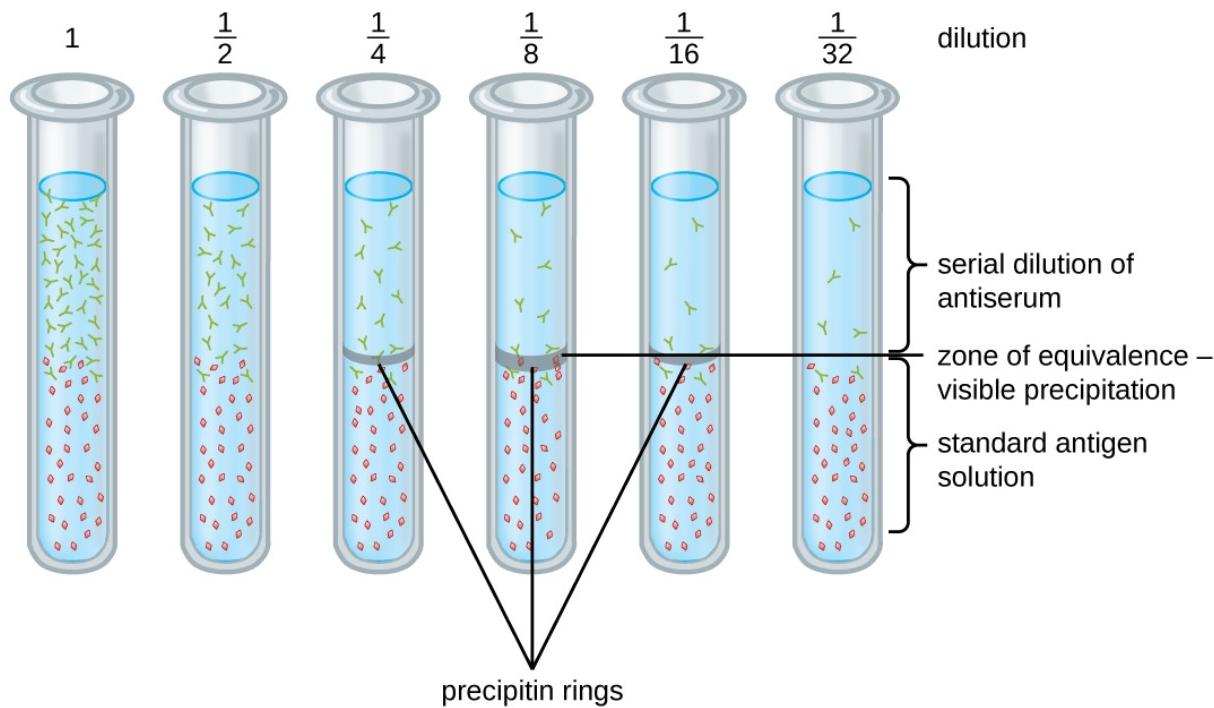
Note:

- What is a precipitin?
- Why do polyclonal antisera produce a better precipitin reaction?

Precipitin Ring Test

A variety of techniques allow us to use precipitin formation to quantify either antigen concentration or the amount of antibody present in an antiserum. One such technique is the **precipitin ring test** ([\[link\]](#)), which is used to determine the relative amount of antigen-specific antibody in a sample of serum. To perform this test, a set of test tubes is prepared by adding an antigen solution to the bottom of each tube. Each tube receives the same volume of solution, and the concentration of antigens is constant (e.g., 1 mg/mL). Next, glycerol is added to the antigen solution in each test tube, followed by a serial dilution of the antiserum. The glycerol prevents mixing of the antiserum with the antigen solution, allowing antigen-antibody binding to take place only at the interface of the two solutions. The result is a visible ring of precipitin in the tubes that have an antigen-antibody ratio within the equivalence zone. This highest dilution with a visible ring is used to determine the **titer** of the antibodies. The titer is the reciprocal of the highest dilution showing a positive result, expressed as a whole number. In [\[link\]](#), the titer is 16.

While a measurement of titer does not tell us in absolute terms how much antibody is present, it does give a measure of biological activity, which is often more important than absolute amount. In this example, it would not be useful to know what mass of IgG were present in the antiserum, because there are many different specificities of antibody present; but it is important for us to know how much of the antibody activity in a patient's serum is directed against the antigen of interest (e.g., a particular pathogen or allergen).



A precipitin ring test is performed using a standard antigen solution in the bottom of the tube and a serial dilution of antiserum in the top of the tube. Glycerol prevents the two solutions from mixing so that precipitation only occurs at the interface. A visible ring of precipitation is seen in the 1/4, 1/8, and 1/16 dilutions, indicating that these concentrations are within the equivalence zone. Since 1/16 is the highest dilution in which a precipitin is observed, the titer is the reciprocal, or 16.

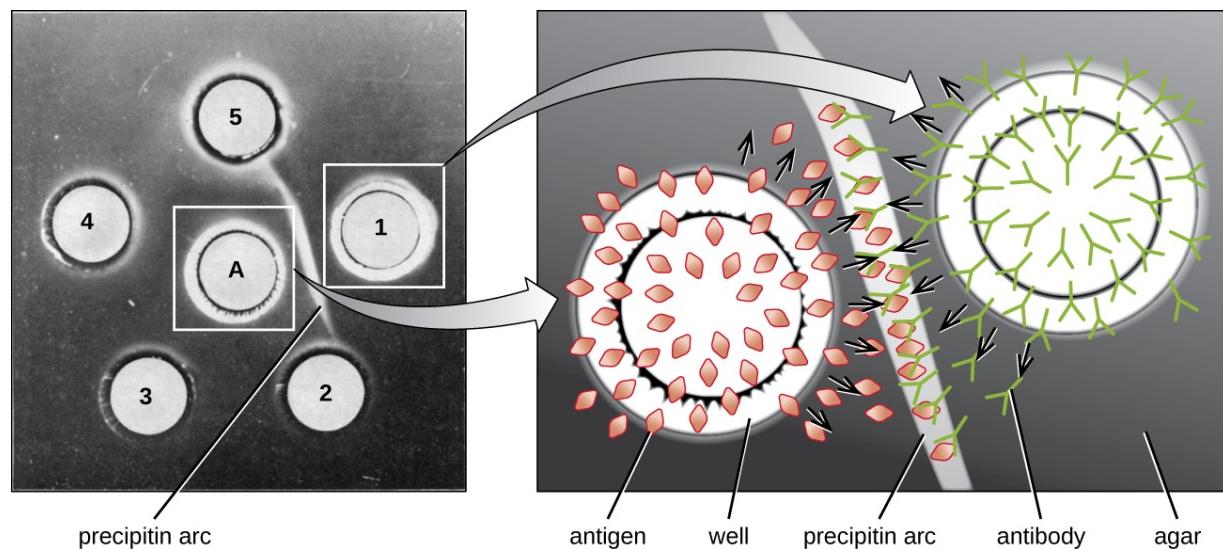
Ouchterlony Assay

While the precipitin ring test provides insights into antibody-antigen interactions, it also has some drawbacks. It requires the use of large amounts of serum, and great care must be taken to avoid mixing the solutions and disrupting the ring. Performing a similar test in an agar gel matrix can minimize these problems. This type of assay is variously called

double immunodiffusion or the **Ouchterlony assay** for Orjan Ouchterlony, [\[footnote\]](#) who first described the technique in 1948. Ouchterlony, Örjan, "In Vitro Method for Testing the Toxin-Producing Capacity of Diphtheria Bacteria," *Acta Pathologica Microbiologica Scandinavica* 26, no. 4 (1949): 516-24.

When agar is highly purified, it produces a clear, colorless gel. Holes are punched in the gel to form wells, and antigen and antisera are added to neighboring wells. Proteins are able to diffuse through the gel, and precipitin arcs form between the wells at the zone of equivalence. Because the precipitin lattice is too large to diffuse through the gel, the arcs are firmly locked in place and easy to see ([\[link\]](#)).

Although there are now more sensitive and quantitative methods of detecting antibody-antigen interactions, the Ouchterlony test provides a rapid and qualitative way of determining whether an antiserum has antibodies against a particular antigen. The Ouchterlony test is particularly useful when looking for cross-reactivity. We can check an antiserum against a group of closely related antigens and see which combinations form precipitin arcs.

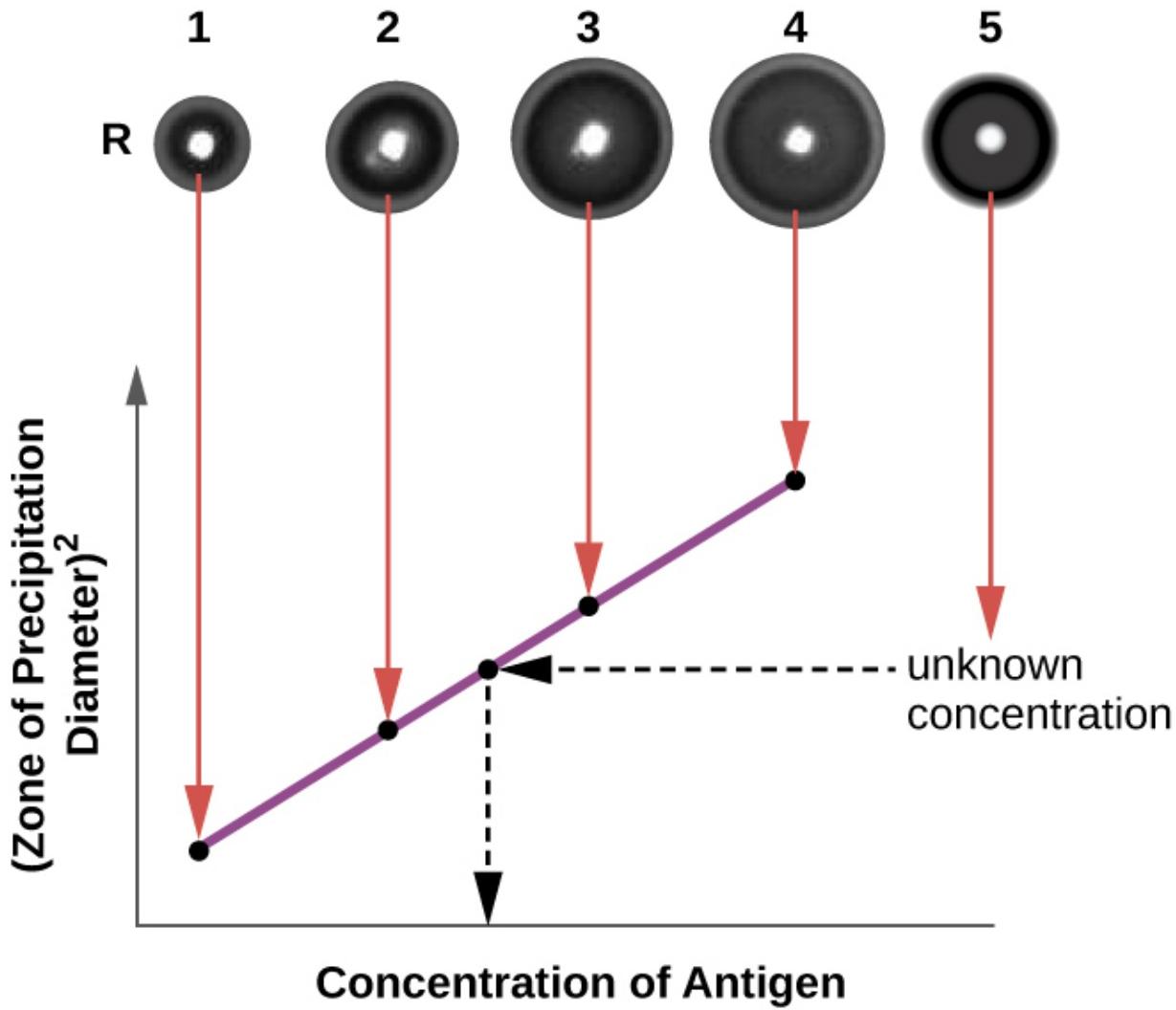


The Ouchterlony test places antigen (well A) and antisera (wells 1 through 5) in a gel. The antibodies and antigen diffuse through the gel,

causing a precipitin arc to form at the zone of equivalence. In this example, only the antiserum in well 1 contains antibodies to the antigen. The resulting precipitin arc is stable because the lattice is too large to diffuse through the gel. (credit left: modification of work by Higgins PJ, Tong C, Borenfreund E, Okin RS, Bendich A)

Radial Immunodiffusion Assay

The **radial immunodiffusion** (RID) assay is similar to the Ouchterlony assay but is used to precisely quantify antigen concentration rather than to compare different antigens. In this assay, the antiserum is added to tempered agar (liquid agar at slightly above 45 °C), which is poured into a small petri dish or onto a glass slide and allowed to cool. Wells are cut in the cooled agar, and antigen is then added to the wells and allowed to diffuse. As the antigen and antibody interact, they form a zone of precipitation. The square of the diameter of the zone of precipitation is directly proportional to the concentration of antigen. By measuring the zones of precipitation produced by samples of known concentration (see the outer ring of samples in [[link](#)]), we can prepare a standard curve for determining the concentration of an unknown solution. The RID assay is also useful test for determining the concentration of many serum proteins such as the C3 and C4 complement proteins, among others.



In this radial immunodiffusion (RID) assay, an antiserum is mixed with the agar before it is cooled, and solutions containing antigen are added to each well in increasing concentrations (wells 1–4). An antigen solution of an unknown concentration is added to well 5. The zones of precipitation are measured and plotted against a standard curve to determine the antigen concentration of the unknown sample. (credit circles: modification of work by Kangwa M, Yelemane V, Polat AN, Gorrepati KD, Grasselli M, Fernández-Lahore M)

Note:

- Why does a precipitin ring form in a precipitin ring test, and what are some reasons why a ring might not form?
- Compare and contrast the techniques used in an Ouchterlony assay and a radial immunodiffusion assay.

Flocculation Assays

A flocculation assay is similar to a precipitin reaction except that it involves insoluble antigens such as lipids. A **flocculant** is similar to a precipitin in that there is a visible lattice of antigen and antibody, but because lipids are insoluble in aqueous solution, they cannot precipitate. Instead of precipitation, flocculation (foaming) is observed in the test tube fluid.

Note:

Using Flocculation to Test for Syphilis

Syphilis is a sexually transmitted infection that can cause severe, chronic disease in adults. In addition, it is readily passed from infected mothers to their newborns during pregnancy and childbirth, often resulting in stillbirth or serious long-term health problems for the infant. Unfortunately, syphilis can also be difficult to diagnose in expectant mothers, because it is often asymptomatic, especially in women. In addition, the causative agent, the bacterium *Treponema pallidum*, is both difficult to grow on conventional lab media and too small to see using routine microscopy. For these reasons, presumptive diagnoses of syphilis are generally confirmed indirectly in the laboratory using tests that detect antibodies to treponemal antigens.

In 1906, German scientist August von Wassermann (1866–1925) introduced the first test for syphilis that relied on detecting anti-treponemal antibodies in the patient's blood. The antibodies detected in the Wassermann test were antiphospholipid antibodies that are nonspecific to *T. pallidum*. Their presence can assist in the diagnosis of syphilis, but because they are nonspecific, they can also lead to false-positive results in

patients with other diseases and autoimmune conditions. The original Wasserman test has been modified over the years to minimize false-positives and is now known as the Venereal Disease Research Lab test, better known by its acronym, the VDRL test.

To perform the VDRL test, patient serum or cerebral spinal fluid is placed on a slide with a mixture of cardiolipin (an antigenic phospholipid found in the mitochondrial membrane of various pathogens), lecithin, and cholesterol. The lecithin and cholesterol stabilize the reaction and diminish false positives. Anti-treponemal antibodies from an infected patient's serum will bind cardiolipin and form a flocculant. Although the VDRL test is more specific than the original Wassermann assay, false positives may still occur in patients with autoimmune diseases that cause extensive cell damage (e.g., systemic lupus erythematosus).

Neutralization Assay

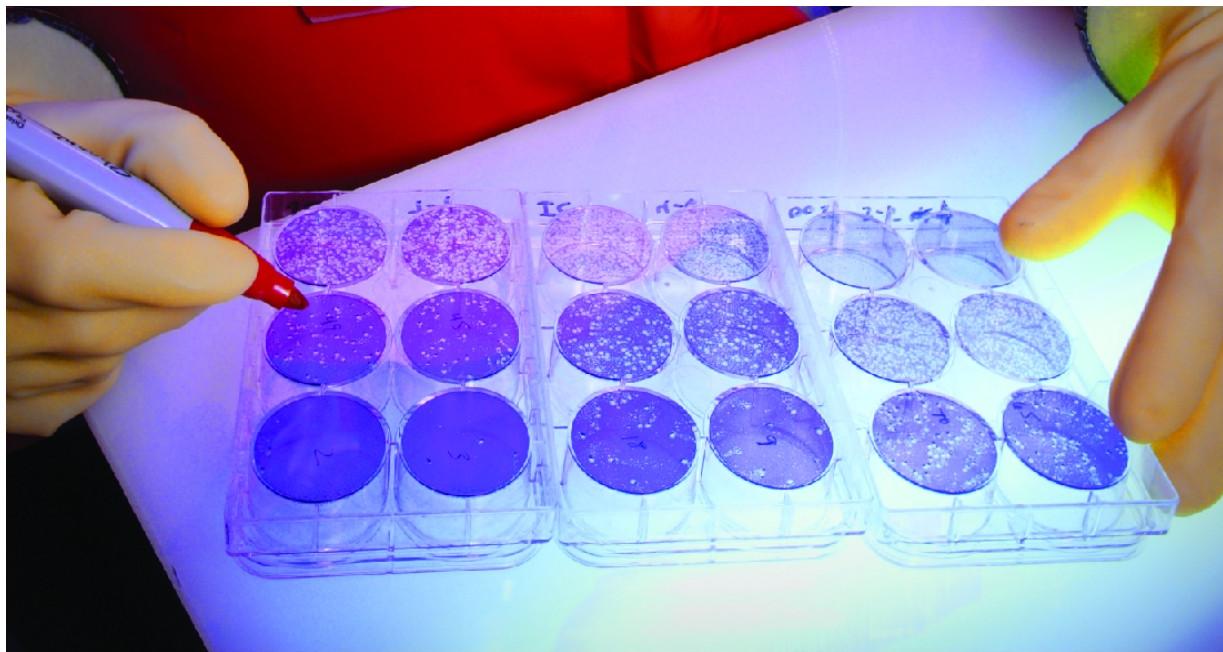
To cause infection, viruses must bind to receptors on host cells. Antiviral antibodies can neutralize viral infections by coating the virions, blocking the binding ([\[link\]](#)). This activity neutralizes virions and can result in the formation of large antibody-virus complexes (which are readily removed by phagocytosis) or by antibody binding to the virus and blocking its binding to host cell receptors. This neutralization activity is the basis of neutralization assays, sensitive assays used for diagnoses of viral infections.

When viruses infect cells, they often cause damage (cytopathic effects) that may include lysis of the host cells. Cytopathic effects can be visualized by growing host cells in a petri dish, covering the cells with a thin layer of agar, and then adding virus (see [Isolation, Culture, and Identification of Viruses](#)). The virus will diffuse very slowly through the agar. A virus will enter a host cell, proliferate (causing cell damage), be released from the dead host cell, and then move to neighboring cells. As more and more cells die, plaques of dead cells will form ([\[link\]](#)).

During the course of a viral infection, the patient will mount an antibody response to the virus, and we can quantify those antibodies using a plaque reduction assay. To perform the assay, a serial dilution is carried out on a

serum sample. Each dilution is then mixed with a standardized amount of the suspect virus. Any virus-specific antibodies in the serum will neutralize some of the virus. The suspensions are then added to host cells in culture to allow any nonneutralized virus to infect the cells and form plaques after several days. The titer is defined as the reciprocal of the highest dilution showing a 50% reduction in plaques. Titer is always expressed as a whole number. For example, if a 1/64 dilution was the highest dilution to show 50% plaque reduction, then the titer is 64.

The presence of antibodies in the patient's serum does not tell us whether the patient is currently infected or was infected in the past. Current infections can be identified by waiting two weeks and testing another serum sample. A four-fold increase in neutralizing titer in this second sample indicates a new infection.



In a neutralization assay, antibodies in patient serum neutralize viruses added to the wells, preventing the formation of plaques. In the assay pictured, the wells with numerous plaques (white patches) contain a low concentration of antibodies. The wells with relatively few plaques

have a high concentration of antibodies. (credit: modification of work by Centers for Disease Control and Prevention)

Note:

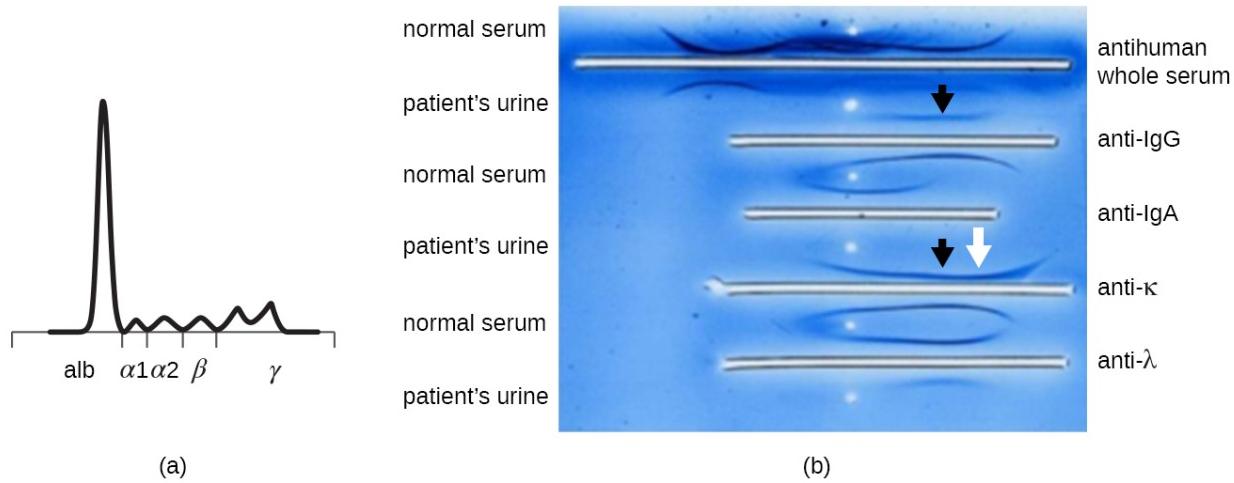
- In a neutralization assay, if a patient's serum has high numbers of antiviral antibodies, would you expect to see more or fewer plaques?

Immunoelectrophoresis

When a patient has elevated protein levels in the blood or is losing protein in the urine, a clinician will often order a polyacrylamide gel electrophoresis (PAGE) assay (see [Visualizing and Characterizing DNA, RNA, and Protein](#)). This assay compares the relative abundance of the various types of serum proteins. Abnormal protein electrophoresis patterns can be further studied using **immunoelectrophoresis (IEP)**. The IEP begins by running a PAGE. Antisera against selected serum proteins are added to troughs running parallel to the electrophoresis track, forming precipitin arcs similar to those seen in an Ouchterlony assay ([\[link\]](#)). This allows the identification of abnormal immunoglobulin proteins in the sample.

IEP is particularly useful in the diagnosis of multiple myeloma, a cancer of antibody-secreting cells. Patients with multiple myeloma cannot produce healthy antibodies; instead they produce abnormal antibodies that are monoclonal proteins (M proteins). Thus, patients with multiple myeloma will present with elevated serum protein levels that show a distinct band in the gamma globulin region of a protein electrophoresis gel and a sharp spike (in M protein) on the densitometer scan rather than the normal broad smear ([\[link\]](#)). When antibodies against the various types of antibody heavy and light chains are used to form precipitin arcs, the M protein will cause

distinctly skewed arcs against one class of heavy chain and one class of light chain as seen in [\[link\]](#).



(a) This graph shows normal measurements of serum proteins. (b) This photograph shows an immunoelectrophoresis of urine. After electrophoresis, antisera were added to the troughs and the precipitin arcs formed, illustrating the distribution of specific proteins. The skewed arcs (arrows) help to diagnose multiple myeloma. (credit a, b: modification of work by Izawa S, Akimoto T, Ikeuchi H, Kusano E, Nagata D)

Note:

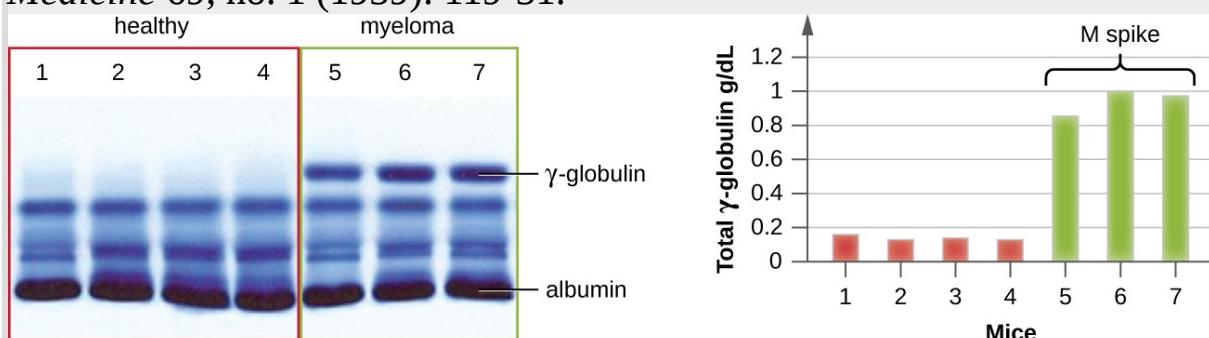
Protein Electrophoresis and the Characterization of Immunoglobulin Structure

The advent of electrophoresis ultimately led to researching and understanding the structure of antibodies. When Swedish biochemist Arne Tiselius (1902–1971) published the first protein electrophoresis results in 1937, [\[footnote\]](#) he could identify the protein albumin (the smallest and most abundant serum protein) by the sharp band it produced in the gel. The other serum proteins could not be resolved in a simple protein

electrophoresis, so he named the three broad bands, with many proteins in each band, alpha, beta, and gamma globulins. Two years later, American immunologist Elvin Kabat (1914–2000) traveled to Sweden to work with Tiselius using this new technique and showed that antibodies migrated as gamma globulins.[\[footnote\]](#) With this new understanding in hand, researchers soon learned that multiple myeloma, because it is a cancer of antibody-secreting cells, could be tentatively diagnosed by the presence of a large M spike in the gamma-globulin region by protein electrophoresis. Prior to this discovery, studies on immunoglobulin structure had been minimal, because of the difficulty of obtaining pure samples to study. Sera from multiple myeloma patients proved to be an excellent source of highly enriched monoclonal immunoglobulin, providing the raw material for studies over the next 20-plus years that resulted in the elucidation of the structure of immunoglobulin.

Tiselius, Arne, “Electrophoresis of Serum Globulin: Electrophoretic Analysis of Normal and Immune Sera,” *Biochemical Journal* 31, no. 9 (1937): 1464.

Tiselius, Arne and Elvin A. Kabat. “An Electrophoretic Study of Immune Sera and Purified Antibody Preparations,” *The Journal of Experimental Medicine* 69, no. 1 (1939): 119-31.



Electrophoresis patterns of myeloma (right) and normal sera (left). The proteins have been stained; when the density of each band is quantified by densitometry, the data produce the bar graph on the right. Both gels show the expected dense band of albumin at the bottom and an abnormal spike in the gamma-globulin region. (credit: modification of work by Soodgupta D, Hurchla MA, Jiang M, Zheleznyak A, Weilbaecher KN, Anderson CJ, Tomasson MH, Shokeen M)

Note:

- In general, what does an immunoelectrophoresis assay accomplish?

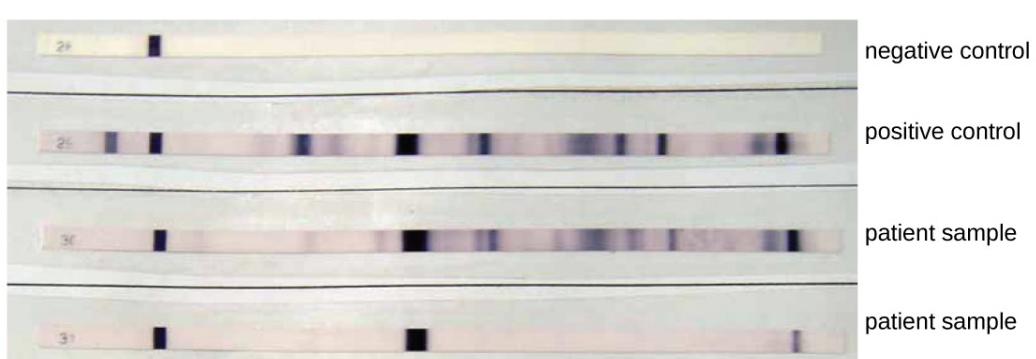
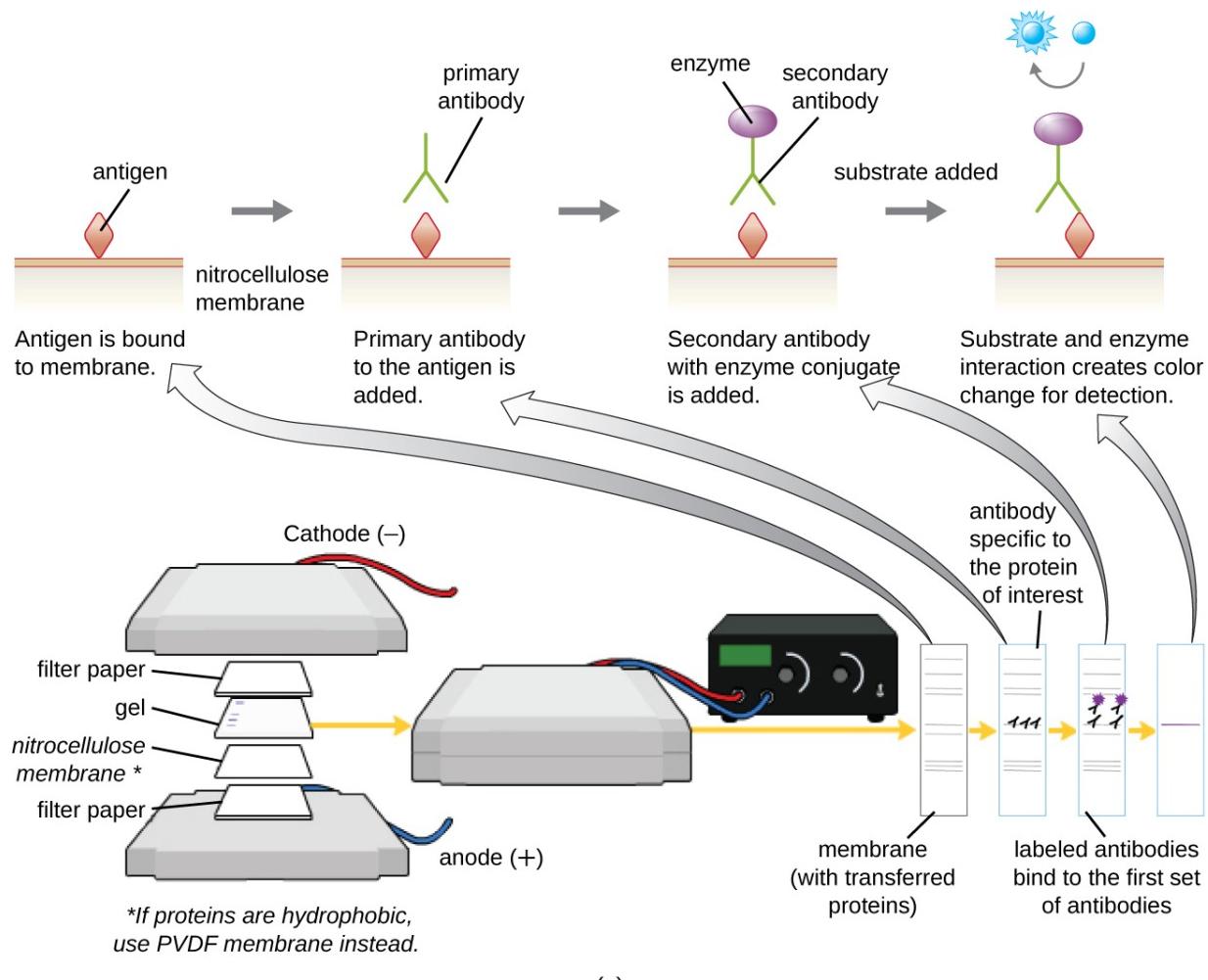
Immunoblot Assay: The Western Blot

After performing protein gel electrophoresis, specific proteins can be identified in the gel using antibodies. This technique is known as the **western blot**. Following separation of proteins by PAGE, the protein antigens in the gel are transferred to and immobilized on a nitrocellulose membrane. This membrane can then be exposed to a primary antibody produced to specifically bind to the protein of interest. A second antibody equipped with a molecular beacon will then bind to the first. These secondary antibodies are coupled to another molecule such as an enzyme or a **fluorophore** (a molecule that fluoresces when excited by light). When using antibodies coupled to enzymes, a **chromogenic substrate** for the enzyme is added. This substrate is usually colorless but will develop color in the presence of the antibody. The fluorescence or substrate coloring identifies the location of the specific protein in the membrane to which the antibodies are bound ([\[link\]](#)).

Typically, polyclonal antibodies are used for western blot assays. They are more sensitive than mAbs because of their ability to bind to various epitopes of the primary antigen, and the signal from polyclonal antibodies is typically stronger than that from mAbs. Monoclonal antibodies can also be used; however, they are much more expensive to produce and are less sensitive, since they are only able to recognize one specific epitope.

Several variations of the western blot are useful in research. In a southwestern blot, proteins are separated by SDS-PAGE, blotted onto a nitrocellulose membrane, allowed to renature, and then probed with a

fluorescently or radioactively labeled DNA probe; the purpose of the southwestern is to identify specific DNA-protein interactions. Far-western blots are carried out to determine protein-protein interactions between immobilized proteins (separated by SDS-PAGE, blotted onto a nitrocellulose membrane, and allowed to renature) and non-antibody protein probes. The bound non-antibody proteins that interact with the immobilized proteins in a far-western blot may be detected by radiolabeling, fluorescence, or the use of an antibody with an enzymatic molecular beacon.



(b)

(a) This diagram summarizes the process of western blotting. Antibodies are used to identify specific bands on the protein gel. (b) A western blot test for antibodies against HIV. The top strip is the negative control; the next strip is the positive control. The bottom two

strips are patient serum samples containing antibodies. (credit a: modification of work by “Bensaccount”/Wikimedia Commons)

Note:

- What is the function of the enzyme in the immunoblot assay?

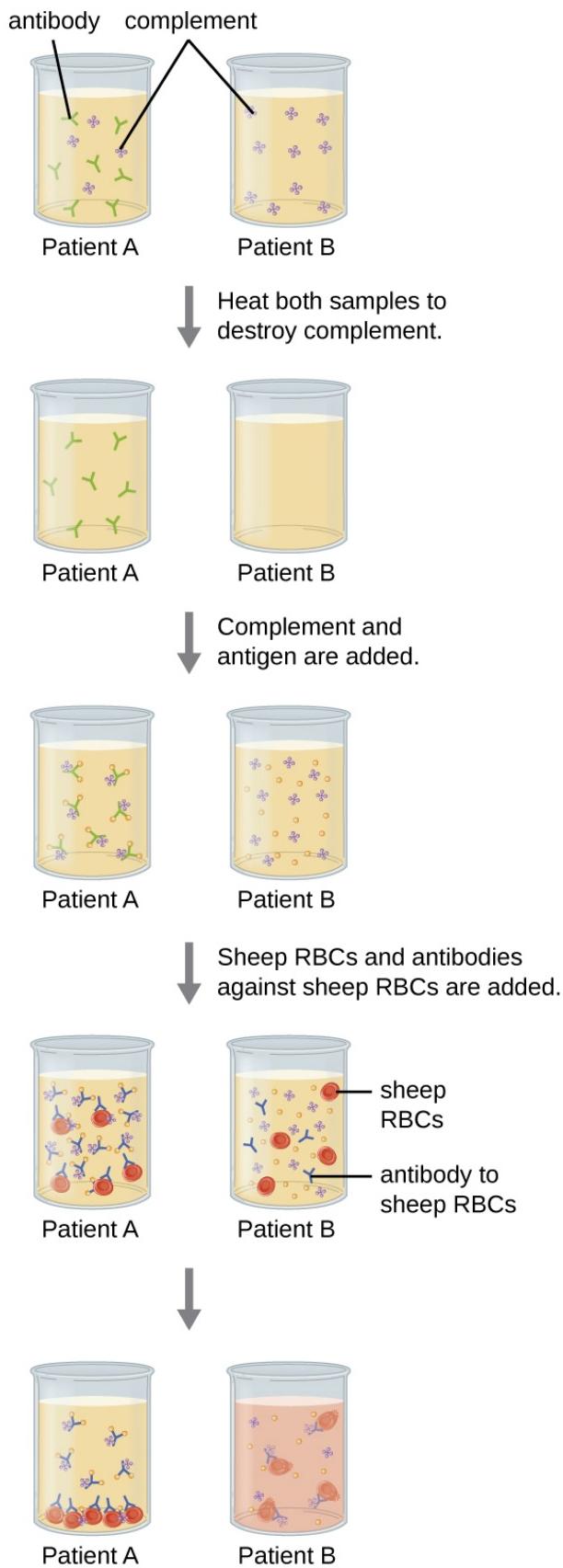
Complement-Mediated Immunoassay

One of the key functions of antibodies is the activation (fixation) of complement. When antibody binds to bacteria, for example, certain complement proteins recognize the bound antibody and activate the complement cascade. In response, other complement proteins bind to the bacteria where some serve as opsonins to increase the efficiency of phagocytosis and others create holes in gram-negative bacterial cell membranes, causing lysis. This lytic activity can be used to detect the presence of antibodies against specific antigens in the serum.

Red blood cells are good indicator cells to use when evaluating complement-mediated cytolysis. Hemolysis of red blood cells releases hemoglobin, which is a brightly colored pigment, and hemolysis of even a small number of red cells will cause the solution to become noticeably pink ([\[link\]](#)). This characteristic plays a role in the **complement fixation test**, which allows the detection of antibodies against specific pathogens. The complement fixation test can be used to check for antibodies against pathogens that are difficult to culture in the lab such as fungi, viruses, or the bacteria *Chlamydia*.

To perform the complement fixation test, antigen from a pathogen is added to patient serum. If antibodies to the antigen are present, the antibody will bind the antigen and fix all the available complement. When red blood cells

and antibodies against red blood cells are subsequently added to the mix, there will be no complement left to lyse the red cells. Thus, if the solution remains clear, the test is positive. If there are no antipathogen antibodies in the patient's serum, the added antibodies will activate the complement to lyse the red cells, yielding a negative test ([\[link\]](#)).



1 Patient A's serum contains antibodies to the suspected antigen. Patient B's serum does not. Both patients have complement, but different amounts.

2 Heating the serum destroys all of the complement in the patient's serum. Antibodies remain in Patient A's serum.

3 An equal amount of complement is then added to the serum for both patients. Antigens are also added. In patient A's serum, antibodies bind to antigens and complement fixation occurs. Patient B's serum lacks antibodies, so complement fixation does not occur.

4 Sheep RBCs and antibodies to sheep RBCs are added to both samples.

5 In patient A, complement is already fixed and cannot lyse RBCs. The antibodies bind to RBCs and settle to the bottom. In patient B, antibodies bind to RBCs and complement lyses the RBCs. Serum turns pink.

The complement fixation test is used to determine whether a patient's serum contains antibodies to a specific antigen. If it does, complement fixation will occur, and there will be no complement available to lyse the antibody-bound sheep red blood cells that are added to the solution in the next step. If the sample does not contain antibodies to the antigen, hemolysis of the sheep blood cells will be observed.

Note:



View this [video](#) to see an outline of the steps of the complement fixation test.

Note:

- In a complement fixation test, if the serum turns pink, does the patient have antibodies to the antigen or not? Explain.

[[link](#)] summarizes the various types of antibody-antigen assays discussed in this section.

Mechanisms of Select Antibody-Antigen Assays

Type of Assay	Mechanism	Examples
Precipitation	Antibody binds to soluble antigen, forming a visible precipitin	Precipitin ring test to visualize lattice formation in solution
		Immunoelectrophoresis to examine distribution of antigens following electrophoresis
		Ouchterlony assay to compare diverse antigens
		Radial immunodiffusion assay to quantify antigens
Flocculation	Antibody binds to insoluble molecules in suspension, forming visible aggregates	VDRL test for syphilis
Neutralization	Antibody binds to virus, blocking viral entry into target cells and preventing formation of plaques	Plaque reduction assay for detecting presence of neutralizing antibodies in patient sera

Mechanisms of Select Antibody-Antigen Assays

Type of Assay	Mechanism	Examples
Complement activation	Antibody binds to antigen, inducing complement activation and leaving no complement to lyse red blood cells	Complement fixation test for patient antibodies against hard-to-culture bacteria such as <i>Chlamydia</i>

Key Concepts and Summary

- When present in the correct ratio, antibody and antigen will form a **precipitin**, or lattice that precipitates out of solution.
- A **precipitin ring test** can be used to visualize lattice formation in solution. The **Ouchterlony assay** demonstrates lattice formation in a gel. The **radial immunodiffusion** assay is used to quantify antigen by measuring the size of a precipitation zone in a gel infused with antibodies.
- Insoluble antigens in suspension will form **flocculants** when bound by antibodies. This is the basis of the VDRL test for syphilis in which anti-treponemal antibodies bind to cardiolipin in suspension.
- Viral infections can be detected by quantifying virus-neutralizing antibodies in a patient's serum.
- Different antibody classes in plasma or serum are identified by using **immunoelectrophoresis**.
- The presence of specific antigens (e.g., bacterial or viral proteins) in serum can be demonstrated by **western blot** assays, in which the proteins are transferred to a nitrocellulose membrane and identified using labeled antibodies.
- In the complement fixation test, complement is used to detect antibodies against various pathogens.

Multiple Choice

Exercise:

Problem:

The formation of _____ is a positive result in the VDRL test.

- a. flocculant
 - b. precipitin
 - c. coagulation
 - d. a bright pink color
-

Solution:

A

Exercise:

Problem:

The titer of a virus neutralization test is the highest dilution of patient serum

- a. in which there is no detectable viral DNA.
 - b. in which there is no detectable viral protein.
 - c. that completely blocks plaque formation.
 - d. that reduces plaque formation by at least 50%.
-

Solution:

D

Exercise:

Problem:

In the Ouchterlony assay, we see a sharp precipitin arc form between antigen and antiserum. Why does this arc remain visible for a long time?

- a. The antibody molecules are too large to diffuse through the agar.
 - b. The precipitin lattice is too large to diffuse through the agar.
 - c. Methanol, added once the arc forms, denatures the protein and blocks diffusion.
 - d. The antigen molecules are chemically coupled to the gel matrix.
-

Solution:

B

Fill in the Blank**Exercise:****Problem:**

When slowly adding antigen to an antiserum, the amount of precipitin would gradually increase until reaching the _____; addition of more antigen after this point would actually decrease the amount of precipitin.

Solution:

equivalence zone or zone of equivalence

Exercise:

Problem:

The radial immunodiffusion test quantifies antigen by mixing _____ into a gel and then allowing antigen to diffuse out from a well cut in the gel.

Solution:

antiserum

Short Answer**Exercise:****Problem:**

Explain why hemolysis in the complement fixation test is a negative test for infection.

Exercise:**Problem:**

What is meant by the term “neutralizing antibodies,” and how can we quantify this effect using the viral neutralization assay?

Critical Thinking**Exercise:****Problem:**

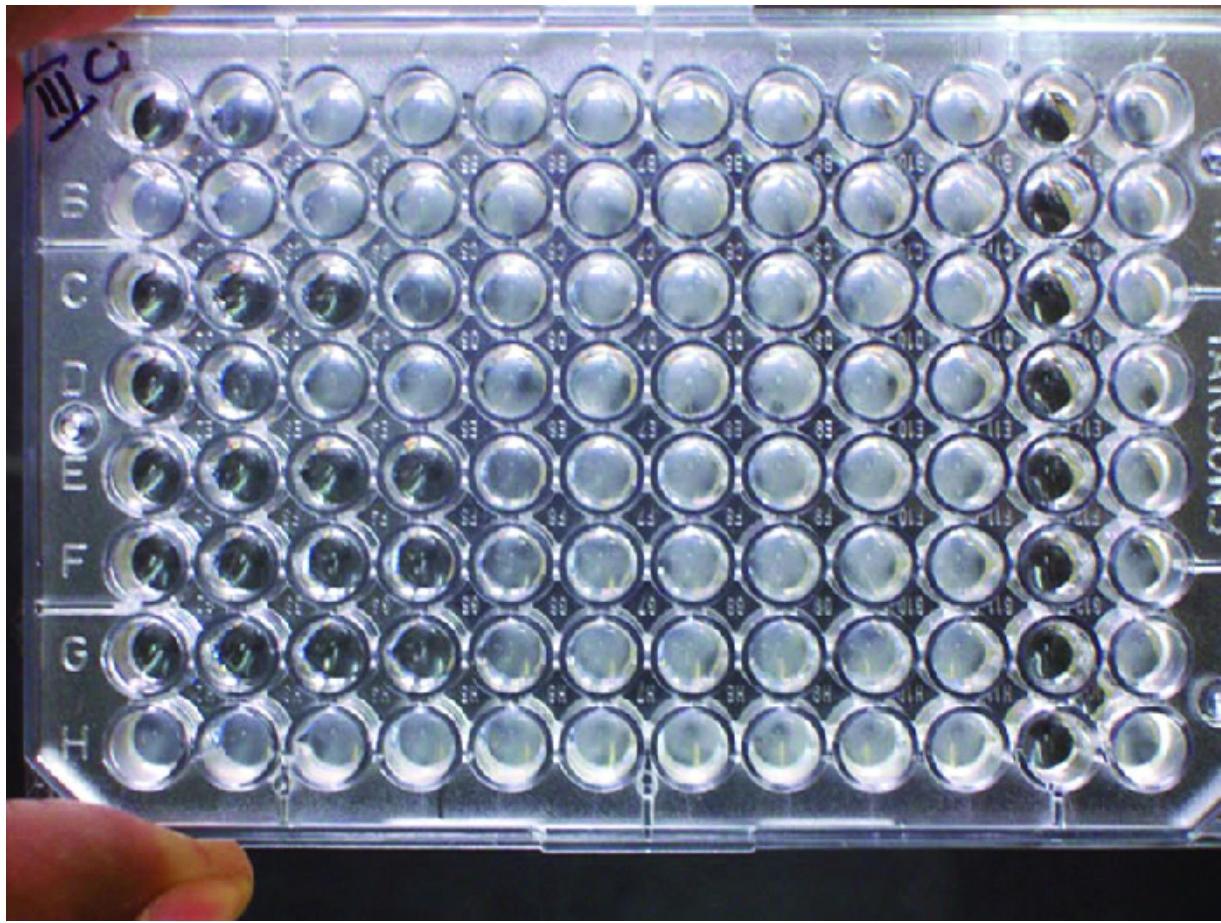
Both IgM and IgG antibodies can be used in precipitation reactions. However, one of these immunoglobulin classes will form precipitates at much lower concentrations than the other. Which class is this, and why is it so much more efficient in this regard?

Agglutination Assays

LEARNING OBJECTIVES

- Compare direct and indirect agglutination
- Identify various uses of hemagglutination in the diagnosis of disease
- Explain how blood types are determined
- Explain the steps used to cross-match blood to be used in a transfusion

In addition to causing precipitation of soluble molecules and flocculation of molecules in suspension, antibodies can also clump together cells or particles (e.g., antigen-coated latex beads) in a process called **agglutination** ([\[link\]](#)). Agglutination can be used as an indicator of the presence of antibodies against bacteria or red blood cells. Agglutination assays are usually quick and easy to perform on a glass slide or **microtiter plate** ([\[link\]](#)). Microtiter plates have an array of wells to hold small volumes of reagents and to observe reactions (e.g., agglutination) either visually or using a specially designed spectrophotometer. The wells come in many different sizes for assays involving different volumes of reagents.



Microtiter plates are used for conducting numerous reactions simultaneously in an array of wells. (credit: modification of work by “Microrao”/Wikimedia)

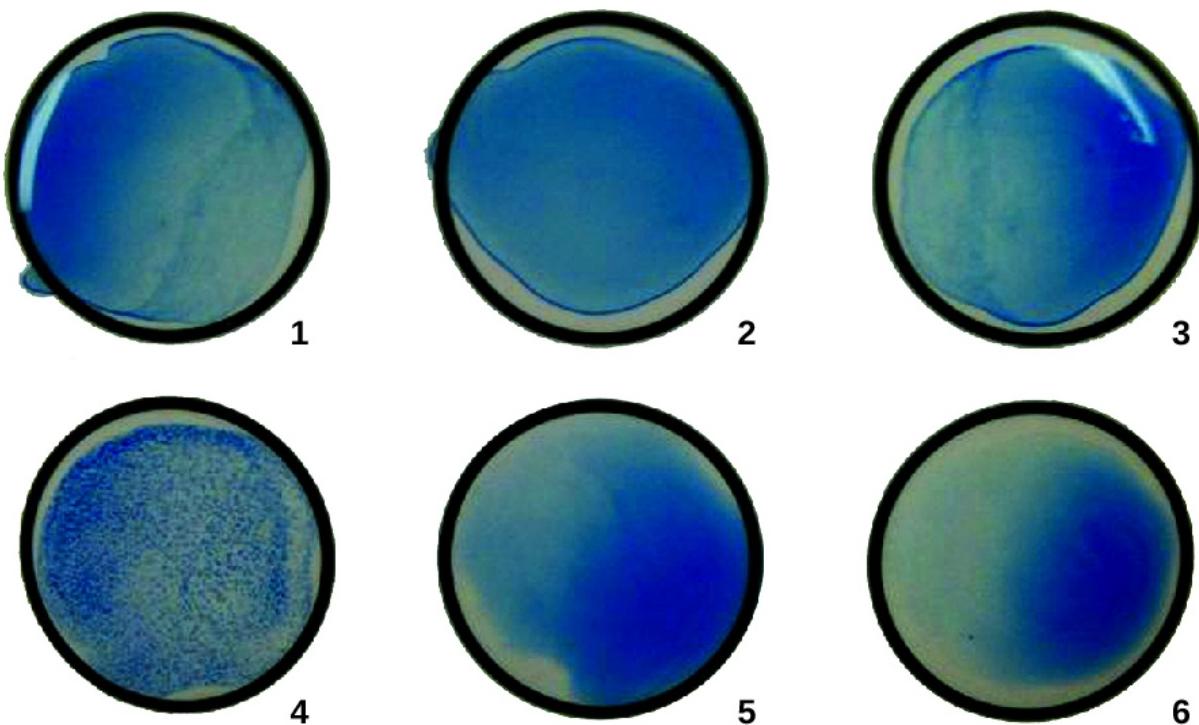
Agglutination of Bacteria and Viruses

The use of agglutination tests to identify streptococcal bacteria was developed in the 1920s by Rebecca Lancefield working with her colleagues A.R. Dochez and Oswald Avery.[\[footnote\]](#) She used antibodies to identify M protein, a virulence factor on streptococci that is necessary for the bacteria’s ability to cause strep throat. Production of antibodies against M protein is crucial in mounting a protective response against the bacteria. Lancefield, Rebecca C., “The Antigenic Complex of *Streptococcus haemoliticus*. I. Demonstration of a Type-Specific Substance in Extracts of

Streptococcus haemolyticus," *The Journal of Experimental Medicine* 47, no. 1 (1928): 91-103.

Lancefield used antisera to show that different strains of the same species of streptococci express different versions of M protein, which explains why children can come down with strep throat repeatedly. Lancefield classified beta-hemolytic streptococci into many groups based on antigenic differences in group-specific polysaccharides located in the bacterial cell wall. The strains are called **serovars** because they are differentiated using antisera. Identifying the serovars present in a disease outbreak is important because some serovars may cause more severe disease than others.

The method developed by Lancefield is a **direct agglutination assay**, since the bacterial cells themselves agglutinate. A similar strategy is more commonly used today when identifying serovars of bacteria and viruses; however, to improve visualization of the agglutination, the antibodies may be attached to inert latex beads. This technique is called an **indirect agglutination assay** (or latex fixation assay), because the agglutination of the beads is a marker for antibody binding to some other antigen ([\[link\]](#)). Indirect assays can be used to detect the presence of either antibodies or specific antigens.



ASM MicrobeLibrary © Hare

Antibodies against six different serovars of Group A strep were attached to latex beads. Each of the six antibody preparations was mixed with bacteria isolated from a patient. The tiny clumps seen in well 4 are indicative of agglutination, which is absent from all other wells. This indicates that the serovar associated with well 4 is present in the patient sample. (credit: modification of work by American Society for Microbiology)

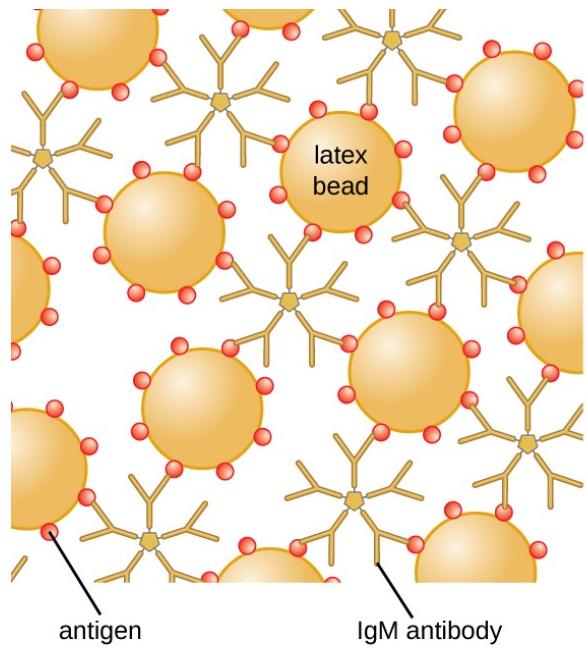
To identify antibodies in a patient's serum, the antigen of interest is attached to latex beads. When mixed with patient serum, the antibodies will bind the antigen, cross-linking the latex beads and causing the beads to agglutinate indirectly; this indicates the presence of the antibody ([\[link\]](#)). This technique is most often used when looking for IgM antibodies, because their structure provides maximum cross-linking. One widely used example of this assay is a test for rheumatoid factor (RF) to confirm a diagnosis of rheumatoid arthritis. RF is, in fact, the presence of IgM antibodies that bind to the patient's own IgG. RF will agglutinate IgG-coated latex beads.

In the reverse test, soluble antigens can be detected in a patient's serum by attaching specific antibodies (commonly mAbs) to the latex beads and mixing this complex with the serum ([\[link\]](#)).

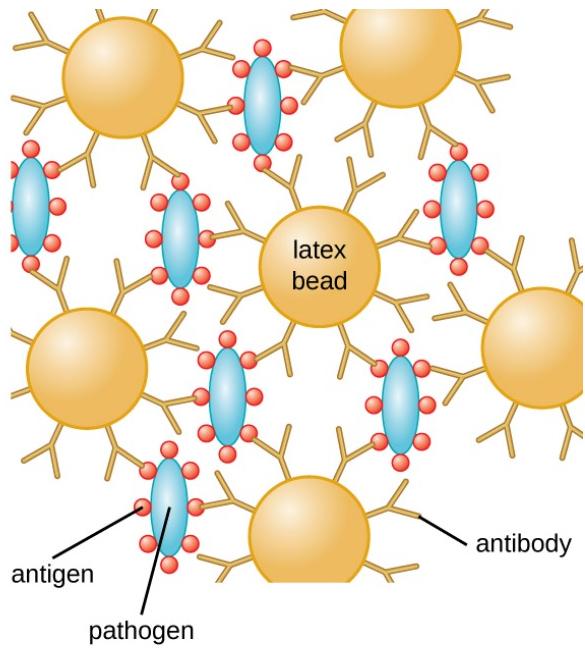
Agglutination tests are widely used in underdeveloped countries that may lack appropriate facilities for culturing bacteria. For example, the Widal test, used for the diagnosis of typhoid fever, looks for agglutination of *Salmonella enterica* subspecies *typhi* in patient sera. The Widal test is rapid, inexpensive, and useful for monitoring the extent of an outbreak; however, it is not as accurate as tests that involve culturing of the bacteria. The Widal test frequently produces false positives in patients with previous infections with other subspecies of *Salmonella*, as well as false negatives in patients with hyperproteinemia or immune deficiencies.

In addition, agglutination tests are limited by the fact that patients generally do not produce detectable levels of antibody during the first week (or longer) of an infection. A patient is said to have undergone **seroconversion** when antibody levels reach the threshold for detection. Typically, seroconversion coincides with the onset of signs and symptoms of disease. However, in an HIV infection, for example, it generally takes 3 weeks for seroconversion to take place, and in some instances, it may take much longer.

Similar to techniques for the precipitin ring test and plaque assays, it is routine to prepare serial two-fold dilutions of the patient's serum and determine the titer of agglutinating antibody present. Since antibody levels change over time in both primary and secondary immune responses, by checking samples over time, changes in antibody titer can be detected. For example, a comparison of the titer during the acute phase of an infection versus the titer from the convalescent phase will distinguish whether an infection is current or has occurred in the past. It is also possible to monitor how well the patient's immune system is responding to the pathogen.



(a) positive agglutination test for antibodies



(b) positive agglutination test for antigens

(a) Latex beads coated with an antigen will agglutinate when mixed with patient serum if the serum contains IgM antibodies against the antigen. (b) Latex beads coated with antibodies will agglutinate when mixed with patient serum if the serum contains antigens specific to the antibodies.

Note:



Watch this [video](#) that demonstrates agglutination reactions with latex beads.

Note:

- How is agglutination used to distinguish serovars from each other?
- In a latex bead assay to test for antibodies in a patient's serum, with what are the beads coated?
- What has happened when a patient has undergone seroconversion?

Hemagglutination

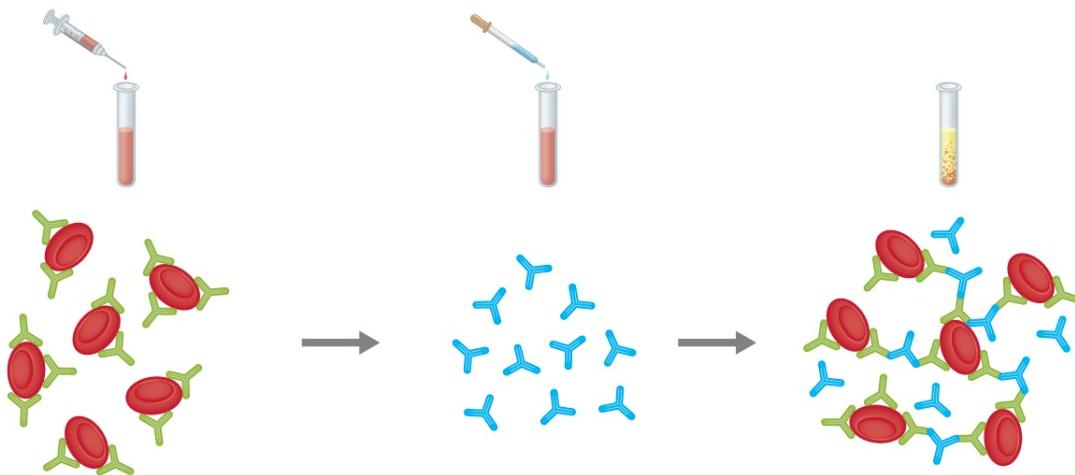
Agglutination of red blood cells is called **hemagglutination**. One common assay that uses hemagglutination is the **direct Coombs' test**, also called the **direct antihuman globulin test (DAT)**, which generally looks for nonagglutinating antibodies. The test can also detect complement attached to red blood cells.

The Coombs' test is often employed when a newborn has jaundice, yellowing of the skin caused by high blood concentrations of bilirubin, a product of the breakdown of hemoglobin in the blood. The Coombs' test is used to determine whether the child's red blood cells have been bound by the mother's antibodies. These antibodies would activate complement, leading to red blood cell lysis and the subsequent jaundice. Other conditions that can cause positive direct Coombs' tests include hemolytic transfusion reactions, autoimmune hemolytic anemia, infectious mononucleosis (caused by Epstein-Barr virus), syphilis, and *Mycoplasma* pneumonia. A positive direct Coombs' test may also be seen in some cancers and as an allergic reaction to some drugs (e.g., penicillin).

The antibodies bound to red blood cells in these conditions are most often IgG, and because of the orientation of the antigen-binding sites on IgG and the comparatively large size of a red blood cell, it is unlikely that any visible agglutination will occur. However, the presence of IgG bound to red blood cells can be detected by adding **Coombs' reagent**, an antiserum

containing antihuman IgG antibodies (that may be combined with anti-complement) ([\[link\]](#)). The Coombs' reagent links the IgG attached to neighboring red blood cells and thus promotes agglutination.

There is also an **indirect Coombs' test** known as the **indirect antiglobulin test (IAT)**. This screens an individual for antibodies against red blood cell antigens (other than the A and B antigens) that are unbound in a patient's serum ([\[link\]](#)). IAT can be used to screen pregnant women for antibodies that may cause hemolytic disease of the newborn. It can also be used prior to giving blood transfusions. More detail on how the IAT is performed is discussed below.

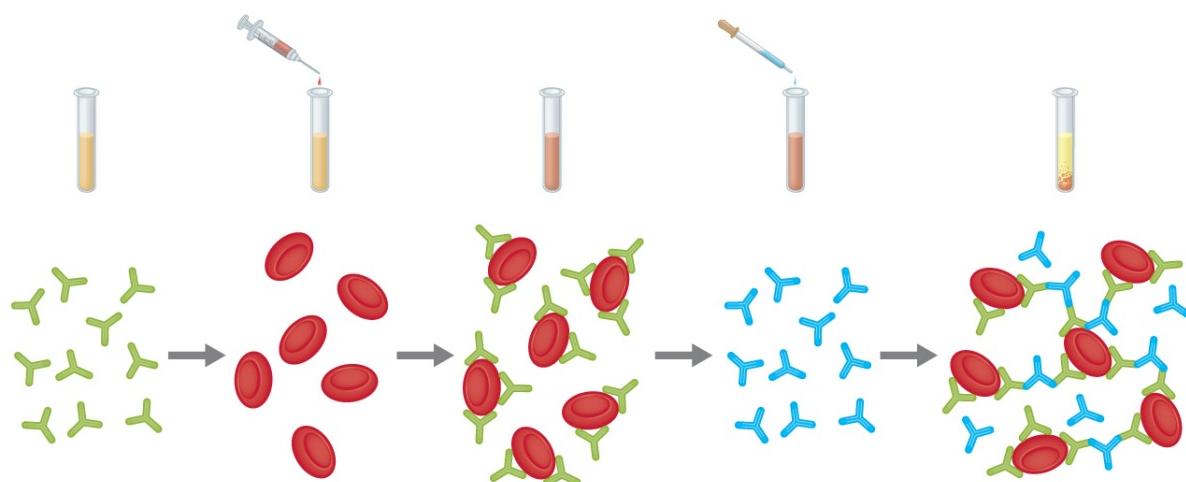


1 Blood sample is taken from a patient with hemolytic anemia with antibodies attached to red blood cells.

2 A few drops of Coombs' reagent (containing antihuman antibodies) is mixed in with the patient's blood sample.

3 Agglutination reaction (clumping) is visible after cross-linkage of antibodies.

Direct Coombs' Test



1 Patient's serum containing antibodies is drawn.

2 Donor blood is added.

3 Patient's antibodies bind to donor's red blood cells.

4 A few drops of Coombs' reagent are mixed with the sample.

5 Agglutination reaction is observed.

Indirect Coombs' Test

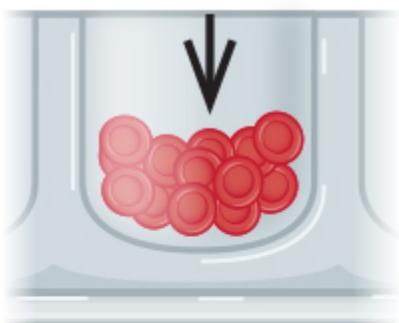
The steps in direct and indirect Coombs' tests are shown in the illustration.

Antibodies that bind to red blood cells are not the only cause of hemagglutination. Some viruses also bind to red blood cells, and this binding can cause agglutination when the viruses cross-link the red blood cells. For example, influenza viruses have two different types of viral spikes called neuraminidase (N) and hemagglutinin (H), the latter named for its ability to agglutinate red blood cells (see [Viruses](#)). Thus, we can use red blood cells to detect the presence of influenza virus by **direct hemagglutination assays** (HA), in which the virus causes visible agglutination of red blood cells. The mumps and rubella viruses can also be detected using HA.

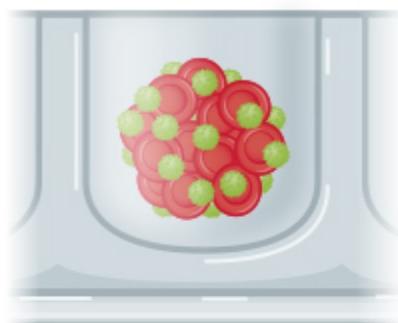
Most frequently, a serial dilution viral agglutination assay is used to measure the titer or estimate the amount of virus produced in cell culture or for vaccine production. A viral titer can be determined using a direct HA by making a serial dilution of the sample containing the virus, starting with a high concentration of sample that is then diluted in a series of wells. The highest dilution producing visible agglutination is the titer. The assay is carried out in a microtiter plate with V- or round-bottomed wells. In the presence of agglutinating viruses, the red blood cells and virus clump together and produce a diffuse mat over the bottom of the well. In the absence of virus, the red blood cells roll or sediment to the bottom of the well and form a dense pellet, which is why flat-bottomed wells cannot be used ([\[link\]](#)).

A modification of the HA assay can be used to determine the titer of antiviral antibodies. The presence of these antibodies in a patient's serum or in a lab-produced antiserum will neutralize the virus and block it from agglutinating the red cells, making this a **viral hemagglutination inhibition assay** (HIA). In this assay, patient serum is mixed with a standardized amount of virus. After a short incubation, a standardized amount of red blood cells is added and hemagglutination is observed. The titer of the patient's serum is the highest dilution that blocks agglutination ([\[link\]](#)).

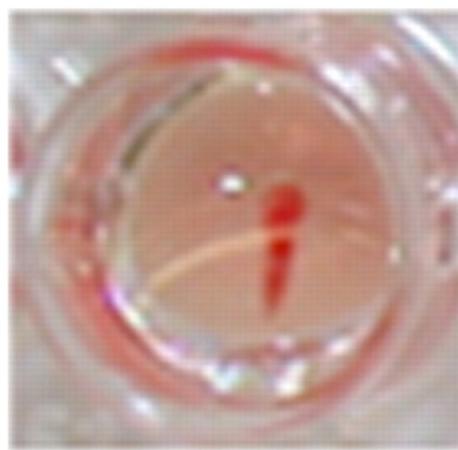
no virus



with virus

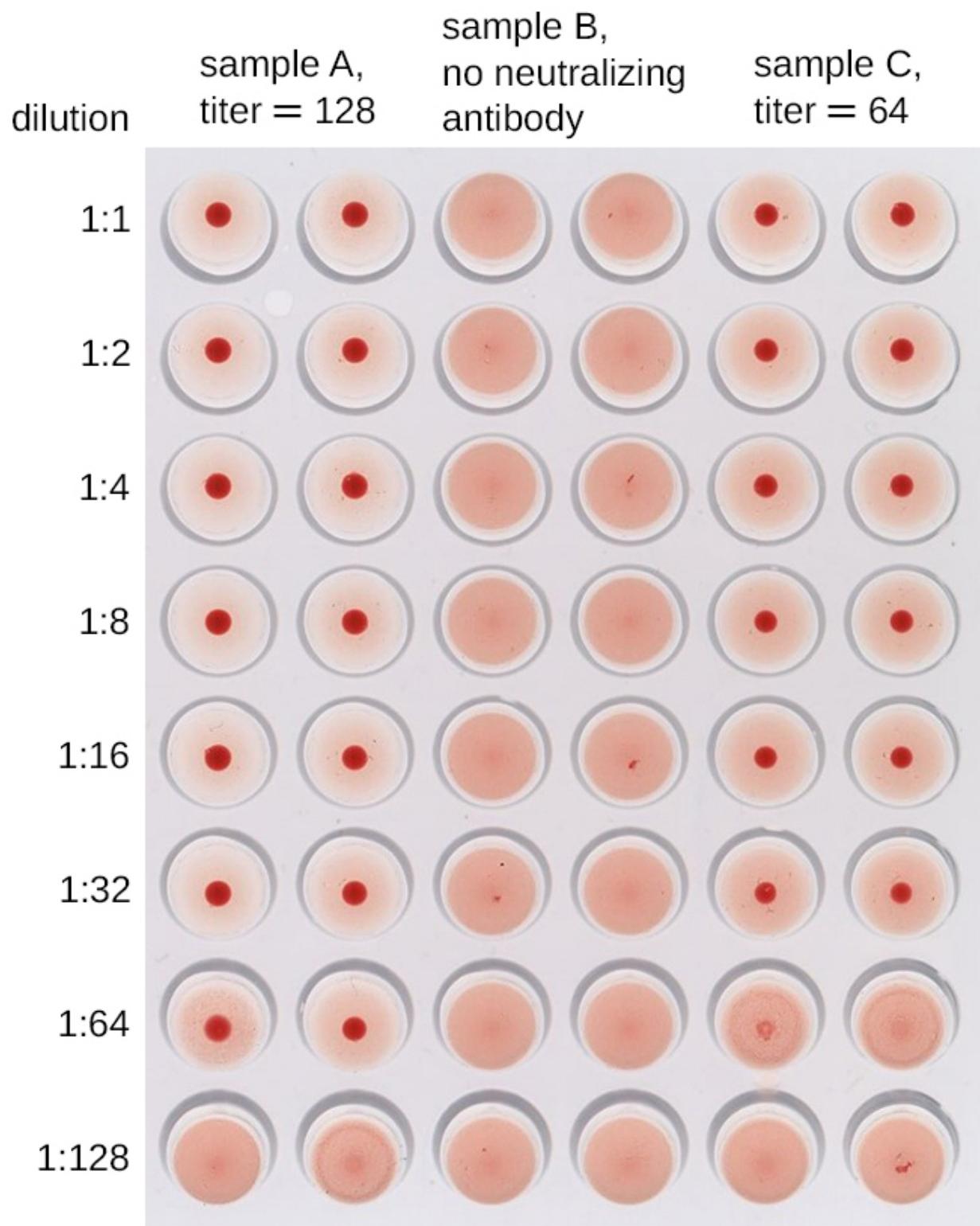


nonagglutinating



agglutinating

A viral suspension is mixed with a standardized amount of red blood cells. No agglutination of red blood cells is visible when the virus is absent, and the cells form a compact pellet at the bottom of the well. In the presence of virus, a diffuse pink precipitate forms in the well. (credit bottom: modification of work by American Society for Microbiology)



In this HIA, serum containing antibodies to influenza virus underwent serial two-fold dilutions in a microtiter plate. Red blood cells were then added to the wells. Agglutination only occurred in those wells

where the antibodies were too dilute to neutralize the virus. The highest concentration at which agglutination occurs is the titer of the antibodies in the patient's serum. In the case of this test, Sample A shows a titer of 128, and Sample C shows a titer of 64. (credit: modification of work by Evan Burkala)

Note:

- What is the mechanism by which viruses are detected in a hemagglutination assay?
- Which hemagglutination result tells us the titer of virus in a sample?

Note:

Animals in the Laboratory

Much of what we know today about the human immune system has been learned through research conducted using animals—primarily, mammals—as models. Besides research, mammals are also used for the production of most of the antibodies and other immune system components needed for immunodiagnostics. Vaccines, diagnostics, therapies, and translational medicine in general have all been developed through research with animal models.

Consider some of the common uses of laboratory animals for producing immune system components. Guinea pigs are used as a source of complement, and mice are the primary source of cells for making mAbs. These mAbs can be used in research and for therapeutic purposes. Antisera are raised in a variety of species, including horses, sheep, goats, and rabbits. When producing an antiserum, the animal will usually be injected at least twice, and adjuvants may be used to boost the antibody response. The larger animals used for making antisera will have blood harvested repeatedly over long periods of time, with little harm to the animals, but that is not usually the case for rabbits. Although we can obtain a few

milliliters of blood from the ear veins of rabbits, we usually need larger volumes, which results in the deaths of the animals.

We also use animals for the study of disease. The only way to grow *Treponema pallidum* for the study of syphilis is in living animals. Many viruses can be grown in cell culture, but growth in cell culture tells us very little about how the immune system will respond to the virus. When working on a newly discovered disease, we still employ Koch's postulates, which require causing disease in lab animals using pathogens from pure culture as a crucial step in proving that a particular microorganism is the cause of a disease. Studying the proliferation of bacteria and viruses in animal hosts, and how the host immune system responds, has been central to microbiological research for well over 100 years.

While the practice of using laboratory animals is essential to scientific research and medical diagnostics, many people strongly object to the exploitation of animals for human benefit. This ethical argument is not a new one—indeed, one of Charles Darwin's daughters was an active antivivisectionist (vivisection is the practice of cutting or dissecting a live animal to study it). Most scientists acknowledge that there should be limits on the extent to which animals can be exploited for research purposes. Ethical considerations have led the National Institutes of Health (NIH) to develop strict regulations on the types of research that may be performed. These regulations also include guidelines for the humane treatment of lab animals, setting standards for their housing, care, and euthanization. The NIH document “Guide for the Care and Use of Laboratory Animals” makes it clear that the use of animals in research is a privilege granted by society to researchers.

The NIH guidelines are based on the principle of the three R's: replace, refine, and reduce. Researchers should strive to *replace* animal models with nonliving models, *replace* vertebrates with invertebrates whenever possible, or use computer-models when applicable. They should *refine* husbandry and experimental procedures to reduce pain and suffering, and use experimental designs and procedures that *reduce* the number of animals needed to obtain the desired information. To obtain funding, researchers must satisfy NIH reviewers that the research justifies the use of animals and that their use is in accordance with the guidelines.

At the local level, any facility that uses animals and receives federal funding must have an Institutional Animal Care and Use Committee

(IACUC) that ensures that the NIH guidelines are being followed. The IACUC must include researchers, administrators, a veterinarian, and at least one person with no ties to the institution, that is, a concerned citizen. This committee also performs inspections of laboratories and protocols. For research involving human subjects, an Institutional Review Board (IRB) ensures that proper guidelines are followed.

Note:



Visit this [site](#) to view the NIH Guide for the Care and Use of Laboratory Animals.

Blood Typing and Cross-Matching

In addition to antibodies against bacteria and viruses to which they have previously been exposed, most individuals also carry antibodies against blood types other than their own. There are presently 33 immunologically important blood-type systems, many of which are restricted within various ethnic groups or rarely result in the production of antibodies. The most important and perhaps best known are the ABO and Rh blood groups (see [\[link\]](#)).

When units of blood are being considered for transfusion, pretransfusion blood testing must be performed. For the blood unit, commercially prepared antibodies against the A, B, and Rh antigens are mixed with red blood cells from the units to initially confirm that the blood type on the unit is accurate.

Once a unit of blood has been requested for transfusion, it is vitally important to make sure the donor (unit of blood) and recipient (patient) are compatible for these crucial antigens. In addition to confirming the blood type of the unit, the patient's blood type is also confirmed using the same commercially prepared antibodies to A, B, and Rh. For example, as shown in [[link](#)], if the donor blood is A-positive, it will agglutinate with the anti-A antiserum and with the anti-Rh antiserum. If no agglutination is observed with any of the sera, then the blood type would be O-negative.

Following determination of the blood type, immediately prior to releasing the blood for transfusion, a **cross-match** is performed in which a small aliquot of the donor red blood cells are mixed with serum from the patient awaiting transfusion. If the patient does have antibodies against the donor red blood cells, hemagglutination will occur. To confirm any negative test results and check for sensitized red blood cells, Coombs' reagent may be added to the mix to facilitate visualization of the antibody-red blood cell interaction.

Under some circumstances, a minor cross-match may be performed as well. In this assay, a small aliquot of donor serum is mixed with patient red blood cells. This allows the detection of agglutinizing antibodies in the donor serum. This test is rarely necessary because transfusions generally use packed red blood cells with most of the plasma removed by centrifugation.

Red blood cells have many other antigens in addition to ABO and Rh. While most people are unlikely to have antibodies against these antigens, women who have had multiple pregnancies or patients who have had multiple transfusions may have them because of repeated exposure. For this reason, an **antibody screen** test is used to determine if such antibodies are present. Patient serum is checked against commercially prepared, pooled, type O red blood cells that express these antigens. If agglutination occurs, the antigen to which the patient is responding must be identified and determined not to be present in the donor unit.



This sample of a commercially produced “bedside” card enables quick typing of both a recipient’s and donor’s blood before transfusion. The card contains three reaction sites or wells. One is coated with an anti-A antibody, one with an anti-B antibody, and one with an anti-Rh antibody. Agglutination of red blood cells in a given site indicates a positive identification of the blood antigens: in this case, A and Rh antigens for blood type A-positive.

Note:

- If a patient's blood agglutinates with anti-B serum, what is the patient's blood type?
- What is a cross-match assay, and why is it performed?

[[link](#)] summarizes the various kinds of agglutination assays discussed in this section.

Mechanisms of Select Antibody-Antigen Assays

Type of Assay	Mechanism	Example
Agglutination	Direct: Antibody is used to clump bacterial cells or other large structures	Serotyping bacteria
	Indirect: Latex beads are coupled with antigen or antibody to look for antibody or antigen, respectively, in patient serum	Confirming the presence of rheumatoid factor (IgM-binding Ig) in patient serum
Hemagglutination	Direct: Some bacteria and viruses cross-link red blood cells and clump them together	Diagnosing influenza, mumps, and measles
	Direct Coombs' test (DAT): Detects nonagglutinating antibodies or complement proteins on red blood cells <i>in vivo</i>	Checking for maternal antibodies binding to neonatal red blood cells
	Indirect Coombs' test (IAT): Screens an individual for antibodies against red blood cell antigens (other than the A and B antigens) that are unbound in a patient's serum <i>in vitro</i>	Performing pretransfusion blood testing

Mechanisms of Select Antibody-Antigen Assays		
Type of Assay	Mechanism	Example
	Viral hemagglutination inhibition: Uses antibodies from a patient to inhibit viral agglutination	Diagnosing various viral diseases by the presence of patient antibodies against the virus
	Blood typing and cross-matching: Detects ABO, Rh, and minor antigens in the blood	Matches donor blood to recipient immune requirements

Key Concepts and Summary

- Antibodies can agglutinate cells or large particles into a visible matrix. **Agglutination** tests are often done on cards or in **microtiter plates** that allow multiple reactions to take place side by side using small volumes of reagents.
- Using antisera against certain proteins allows identification of **serovars** within species of bacteria.
- Detecting antibodies against a pathogen can be a powerful tool for diagnosing disease, but there is a period of time before patients go through **seroconversion** and the level of antibodies becomes detectable.
- Agglutination of latex beads in **indirect agglutination assays** can be used to detect the presence of specific antigens or specific antibodies in patient serum.
- The presence of some antibacterial and antiviral antibodies can be confirmed by the use of the direct **Coombs' test**, which uses Coombs'

reagent to cross-link antibodies bound to red blood cells and facilitate **hemagglutination**.

- Some viruses and bacteria will bind and agglutinate red blood cells; this interaction is the basis of the **direct hemagglutination assay**, most often used to determine the titer of virus in solution.
- **Neutralization assays** quantify the level of virus-specific antibody by measuring the decrease in hemagglutination observed after mixing patient serum with a standardized amount of virus.
- Hemagglutination assays are also used to screen and **cross-match** donor and recipient blood to ensure that the transfusion recipient does not have antibodies to antigens in the donated blood.

Multiple Choice

Exercise:

Problem:

We use antisera to distinguish between various _____ within a species of bacteria.

- isotypes
- serovars
- subspecies
- lines

Solution:

B

Exercise:

Problem:

When using antisera to characterize bacteria, we will often link the antibodies to _____ to better visualize the agglutination.

- latex beads

-
- b. red blood cells
 - c. other bacteria
 - d. white blood cells
-

Solution:

A

Exercise:

Problem:

The antibody screening test that is done along with pretransfusion blood typing is used to ensure that the recipient

- a. does not have a previously undetected bacterial or viral infection.
 - b. is not immunocompromised.
 - c. actually does have the blood type stated in the online chart.
 - d. is not making antibodies against antigens outside the ABO or Rh systems.
-

Solution:

D

Exercise:

Problem:

The direct Coombs' test is designed to detect when people have a disease that causes them to

- a. have an excessively high fever.
 - b. quit making antibodies.
 - c. make too many red blood cells.
 - d. produce antibodies that bind to their own red blood cells.
-

Solution:

D

Exercise:

Problem:

Viral hemagglutination assays only work with certain types of viruses because

- a. the virus must be able to cross-link red blood cells directly.
 - b. the virus must be able to lyse red blood cells.
 - c. the virus must not be able to lyse red blood cells.
 - d. other viruses are too dangerous to work with in a clinical lab setting.
-

Solution:

A

Fill in the Blank

Exercise:

Problem:

In the major cross-match, we mix _____ with the donor red blood cells and look for agglutination.

Solution:

patient serum

Exercise:

Problem:

Coombs' reagent is an antiserum with antibodies that bind to human _____.

Solution:

immunoglobulins/antibodies and/or complement

Short Answer**Exercise:****Problem:**

Explain why the titer of a direct hemagglutination assay is the highest dilution that still causes hemagglutination, whereas in the viral hemagglutination inhibition assay, the titer is the highest dilution at which hemagglutination is not observed.

Exercise:**Problem:**

Why would a doctor order a direct Coombs' test when a baby is born with jaundice?

Critical Thinking**Exercise:****Problem:**

When shortages of donated blood occur, O-negative blood may be given to patients, even if they have a different blood type. Why is this the case? If O-negative blood supplies were depleted, what would be the next-best choice for a patient with a different blood type in critical need of a transfusion? Explain your answers.

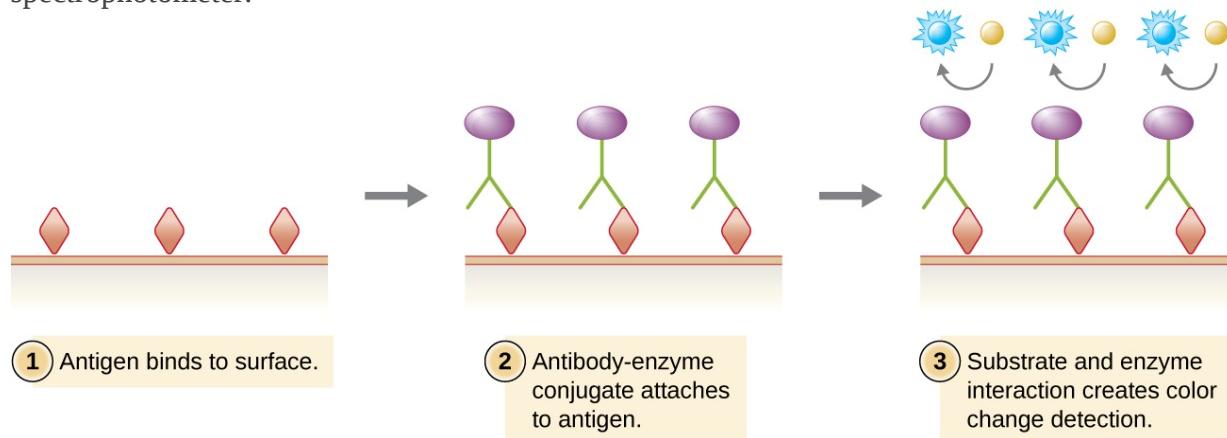
EIA and ELISAs

LEARNING OBJECTIVES

- Explain the differences and similarities between EIA, FEIA, and ELISA
- Describe the difference and similarities between immunohistochemistry and immunocytochemistry
- Describe the different purposes of direct and indirect ELISA

Similar to the western blot, **enzyme immunoassays (EIAs)** use antibodies to detect the presence of antigens. However, EIAs differ from western blots in that the assays are conducted in microtiter plates or *in vivo* rather than on an absorbent membrane. There are many different types of EIAs, but they all involve an antibody molecule whose constant region binds an enzyme, leaving the variable region free to bind its specific antigen. The addition of a substrate for the enzyme allows the antigen to be visualized or quantified ([\[link\]](#)).

In EIAs, the substrate for the enzyme is most often a chromogen, a colorless molecule that is converted into a colored end product. The most widely used enzymes are alkaline phosphatase and horseradish peroxidase for which appropriate substrates are readily available. In some EIAs, the substrate is a **fluorogen**, a nonfluorescent molecule that the enzyme converts into a fluorescent form. EIAs that utilize a fluorogen are called **fluorescent enzyme immunoassays (FEIAs)**. Fluorescence can be detected by either a fluorescence microscope or a spectrophotometer.



Enzyme immunoassays, such as the direct ELISA shown here, use an enzyme-antibody conjugate to deliver a detectable substrate to the site of an antigen. The substrate may be a colorless molecule that is converted into a colored end product or an inactive fluorescent

molecule that fluoresces after enzyme activation. (credit: modification of work by “Cavitri”/Wikimedia Commons)

Note:

The MMR Titer

The MMR vaccine is a combination vaccine that provides protection against measles, mumps, and rubella (German measles). Most people receive the MMR vaccine as children and thus have antibodies against these diseases. However, for various reasons, even vaccinated individuals may become susceptible to these diseases again later in life. For example, some children may receive only one round of the MMR vaccine instead of the recommended two. In addition, the titer of protective antibodies in an individual’s body may begin to decline with age or as the result of some medical conditions.

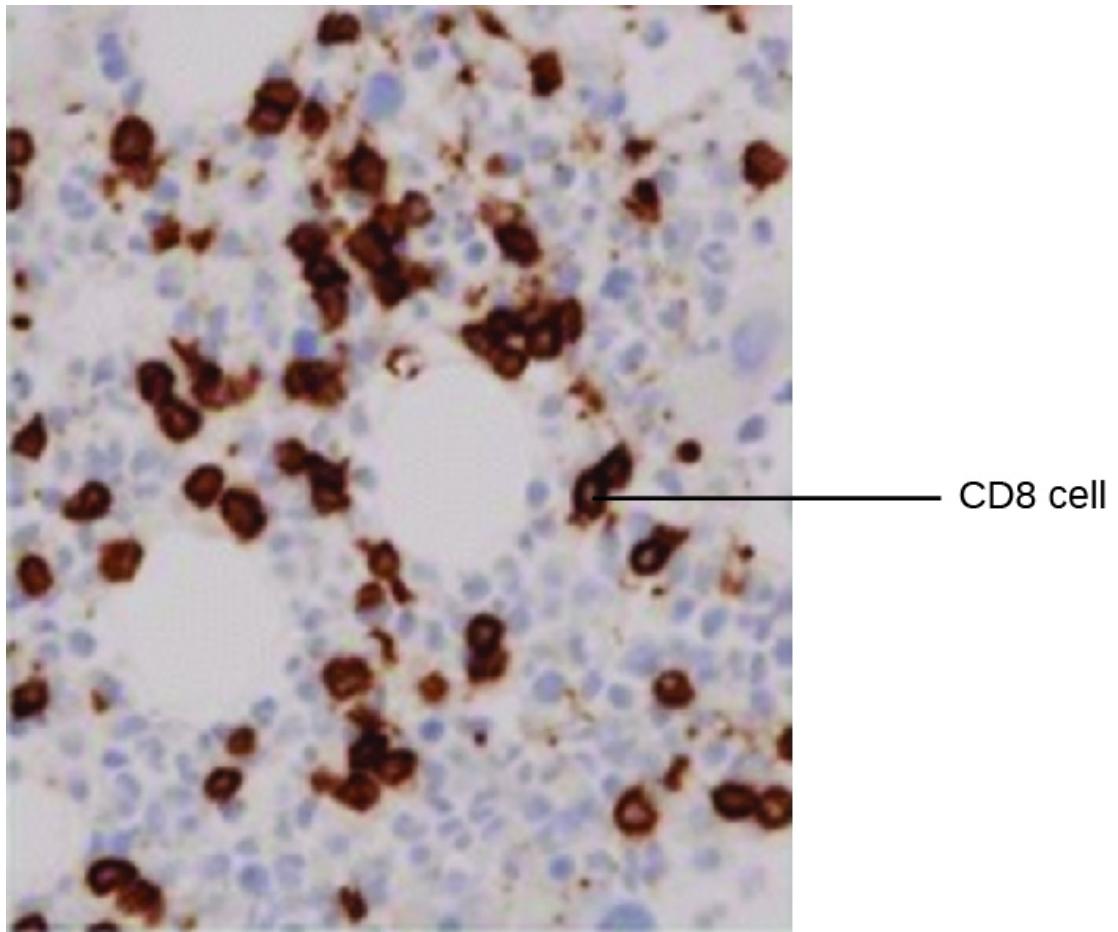
To determine whether the titer of antibody in an individual’s bloodstream is sufficient to provide protection, an MMR titer test can be performed. The test is a simple immunoassay that can be done quickly with a blood sample. The results of the test will indicate whether the individual still has immunity or needs another dose of the MMR vaccine.

Submitting to an MMR titer is often a pre-employment requirement for healthcare workers, especially those who will frequently be in contact with young children or immunocompromised patients. Were a healthcare worker to become infected with measles, mumps, or rubella, the individual could easily pass these diseases on to susceptible patients, leading to an outbreak. Depending on the results of the MMR titer, healthcare workers might need to be revaccinated prior to beginning work.

Immunostaining

One powerful use of EIA is **immunostaining**, in which antibody-enzyme conjugates enhance microscopy. **Immunohistochemistry (IHC)** is used for examining whole tissues. As seen in [\[link\]](#), a section of tissue can be stained to visualize the various cell types. In this example, a mAb against CD8 was used to stain CD8 cells in a section of tonsil tissue. It is now possible to count the number of CD8 cells, determine their relative numbers versus the other cell types present, and determine the location of these cells within this tissue. Such data would be useful for studying diseases such as AIDS, in which the normal function of CD8 cells is crucial for slowing disease progression.

Immunocytochemistry (ICC) is another valuable form of immunostaining. While similar to IHC, in ICC, extracellular matrix material is stripped away, and the cell membrane is etched with alcohol to make it permeable to antibodies. This allows antibodies to pass through the cell membrane and bind to specific targets inside the cell. Organelles, cytoskeletal components, and other intracellular structures can be visualized in this way. While some ICC techniques use EIA, the enzyme can be replaced with a fluorescent molecule, making it a fluorescent immunoassay.



Enzyme-linked antibodies against CD8 were used to stain the CD8 cells in this preparation of bone marrow using a chromogen. (credit: modification of work by Yamashita M, Fujii Y, Ozaki K, Urano Y, Iwasa M, Nakamura S, Fujii S, Abe M, Sato Y, Yoshino T)

Note:

- What is the difference between immunohistochemistry and immunocytochemistry?
- What must be true of the product of the enzymatic reaction used in immunohistochemistry?

Enzyme-linked Immunosorbent Assays (ELISAs)

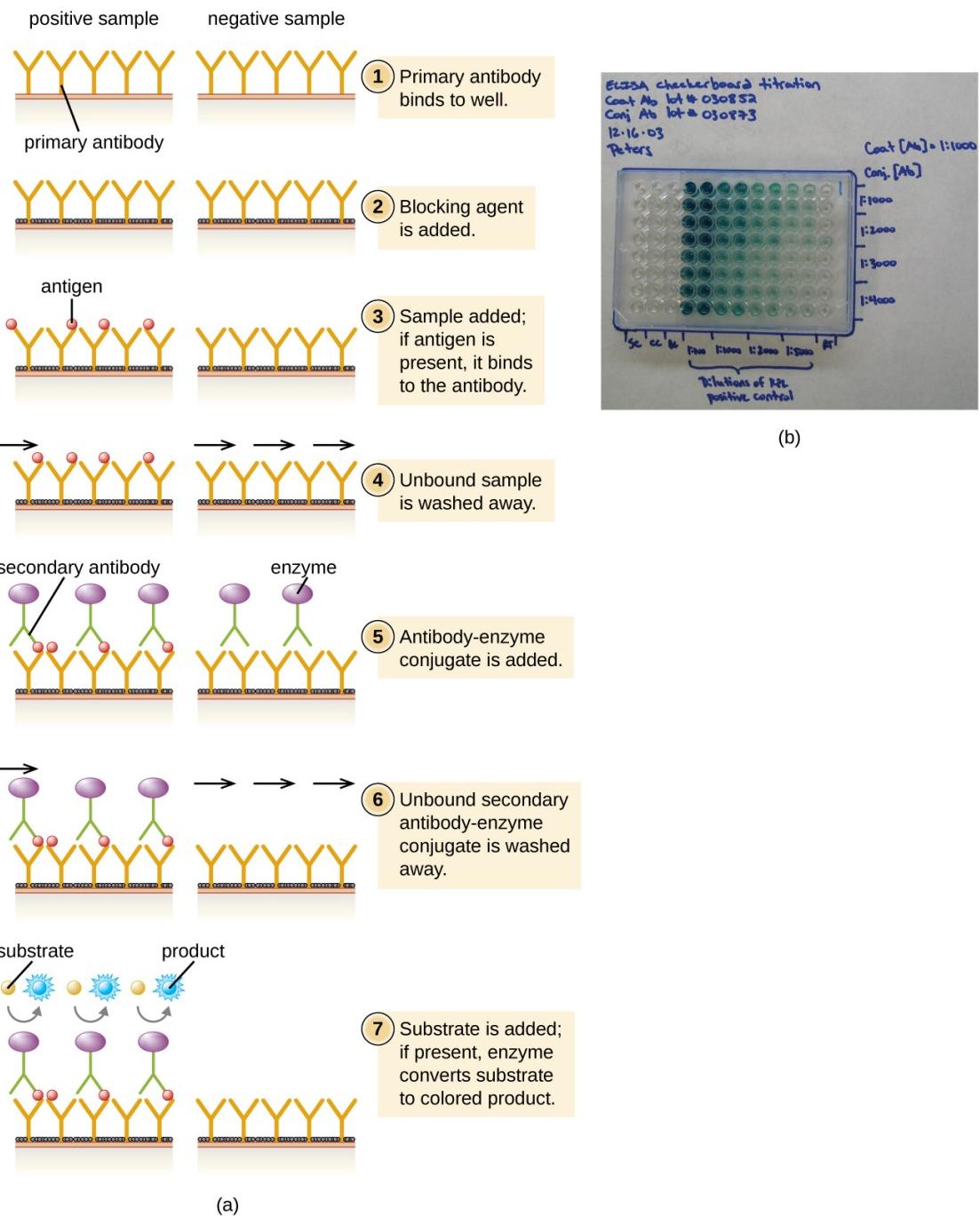
The **enzyme-linked immunosorbent assays (ELISAs)** are widely used EIAs. In the **direct ELISA**, antigens are immobilized in the well of a microtiter plate. An antibody that is specific

for a particular antigen and is conjugated to an enzyme is added to each well. If the antigen is present, then the antibody will bind. After washing to remove any unbound antibodies, a colorless substrate (chromogen) is added. The presence of the enzyme converts the substrate into a colored end product ([\[link\]](#)). While this technique is faster because it only requires the use of one antibody, it has the disadvantage that the signal from a direct ELISA is lower (lower sensitivity).

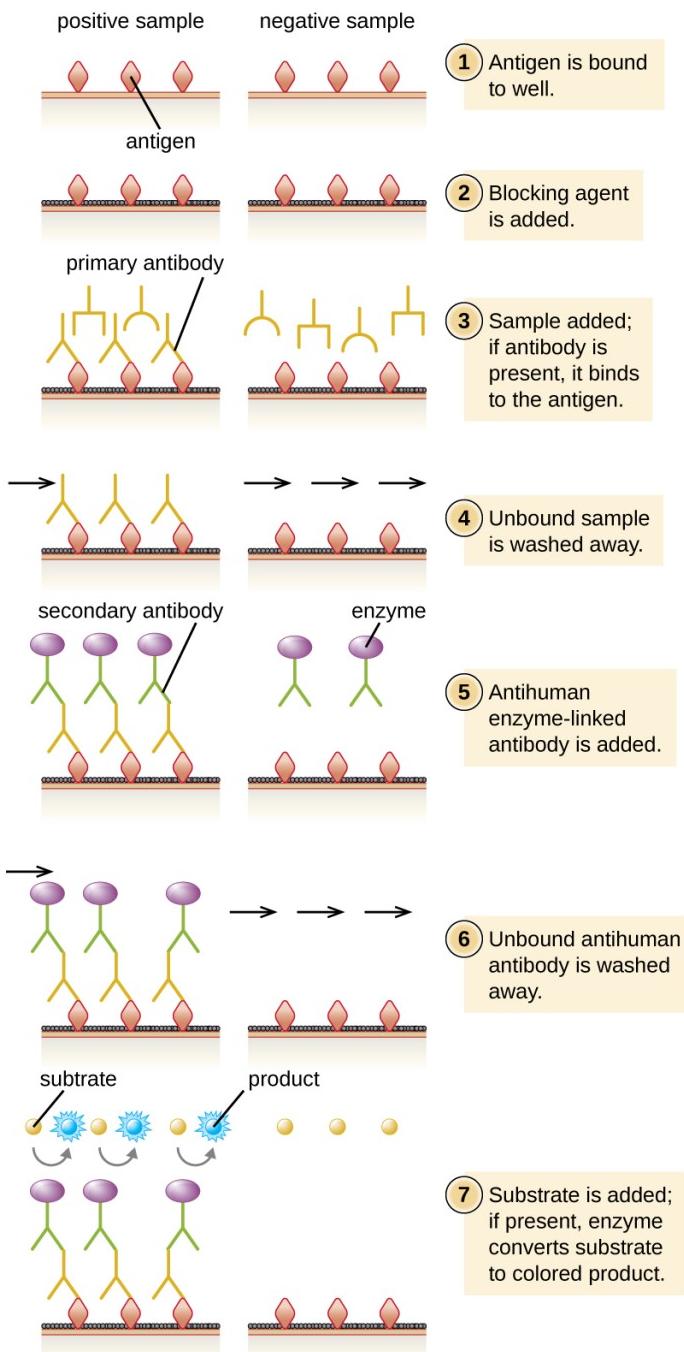
In a **sandwich ELISA**, the goal is to use antibodies to precisely quantify specific antigen present in a solution, such as antigen from a pathogen, a serum protein, or a hormone from the blood or urine to list just a few examples. The first step of a sandwich ELISA is to add the **primary antibody** to all the wells of a microtiter plate ([\[link\]](#)). The antibody sticks to the plastic by hydrophobic interactions. After an appropriate incubation time, any unbound antibody is washed away. Comparable washes are used between each of the subsequent steps to ensure that only specifically bound molecules remain attached to the plate. A blocking protein is then added (e.g., albumin or the milk protein casein) to bind the remaining nonspecific protein-binding sites in the well. Some of the wells will receive known amounts of antigen to allow the construction of a standard curve, and unknown antigen solutions are added to the other wells. The primary antibody captures the antigen and, following a wash, the **secondary antibody** is added, which is a polyclonal antibody that is conjugated to an enzyme. After a final wash, a colorless substrate (chromogen) is added, and the enzyme converts it into a colored end product. The color intensity of the sample caused by the end product is measured with a spectrophotometer. The amount of color produced (measured as absorbance) is directly proportional to the amount of enzyme, which in turn is directly proportional to the captured antigen. ELISAs are extremely sensitive, allowing antigen to be quantified in the nanogram (10^{-9} g) per mL range.

In an **indirect ELISA**, we quantify antigen-specific antibody rather than antigen. We can use indirect ELISA to detect antibodies against many types of pathogens, including *Borrelia burgdorferi* (Lyme disease) and HIV. There are three important differences between indirect and direct ELISAs as shown in [\[link\]](#). Rather than using antibody to capture antigen, the indirect ELISA starts with attaching known antigen (e.g., peptides from HIV) to the bottom of the microtiter plate wells. After blocking the unbound sites on the plate, patient serum is added; if antibodies are present (primary antibody), they will bind the antigen. After washing away any unbound proteins, the secondary antibody with its conjugated enzyme is directed against the primary antibody (e.g., antihuman immunoglobulin). The secondary antibody allows us to quantify how much antigen-specific antibody is present in the patient's serum by the intensity of the color produced from the conjugated enzyme-chromogen reaction.

As with several other tests for antibodies discussed in this chapter, there is always concern about cross-reactivity with antibodies directed against some other antigen, which can lead to false-positive results. Thus, we cannot definitively diagnose an HIV infection (or any other type of infection) based on a single indirect ELISA assay. We must confirm any suspected positive test, which is most often done using either an immunoblot that actually identifies the presence of specific peptides from the pathogen or a test to identify the nucleic acids associated with the pathogen, such as reverse transcriptase PCR (RT-PCR) or a nucleic acid antigen test.



(a) In a sandwich ELISA, a primary antibody is used to first capture an antigen with the primary antibody. A secondary antibody conjugated to an enzyme that also recognizes epitopes on the antigen is added. After the addition of the chromogen, a spectrophotometer measures the absorbance of end product, which is directly proportional to the amount of captured antigen. (b) An ELISA plate shows dilutions of antibodies (left) and antigens (bottom). Higher concentrations result in a darker final color. (credit b: modification of work by U.S. Fish and Wildlife Service Pacific Region)



The indirect ELISA is used to quantify antigen-specific antibodies in patient serum for disease diagnosis. Antigen from the suspected disease agent is attached to microtiter plates. The primary antibody comes from the patient's serum, which

is subsequently bound by the enzyme-conjugated secondary antibody. Measuring the production of end product allows us to detect or quantify the amount of antigen-specific antibody present in the patient's serum.

Note:

- What is the purpose of the secondary antibody in a direct ELISA?
- What do the direct and indirect ELISAs quantify?

Note:

Part 2

Although contacting and testing the 1300 patients for HIV would be time consuming and expensive, administrators hoped to minimize the hospital's liability by proactively seeking out and treating potential victims of the rogue employee's crime. Early detection of HIV is important, and prompt treatment can slow the progression of the disease.

There are a variety of screening tests for HIV, but the most widely used is the indirect ELISA. As with other indirect ELISAs, the test works by attaching antigen (in this case, HIV peptides) to a well in a 96-well plate. If the patient is HIV positive, anti-HIV antibodies will bind to the antigen and be identified by the second antibody-enzyme conjugate.

- How accurate is an indirect ELISA test for HIV, and what factors could impact the test's accuracy?
- Should the hospital use any other tests to confirm the results of the indirect ELISA?

Jump to the [previous](#) Clinical Focus box. Jump to the [next](#) Clinical Focus box.

Immunofiltration and Immunochromatographic Assays

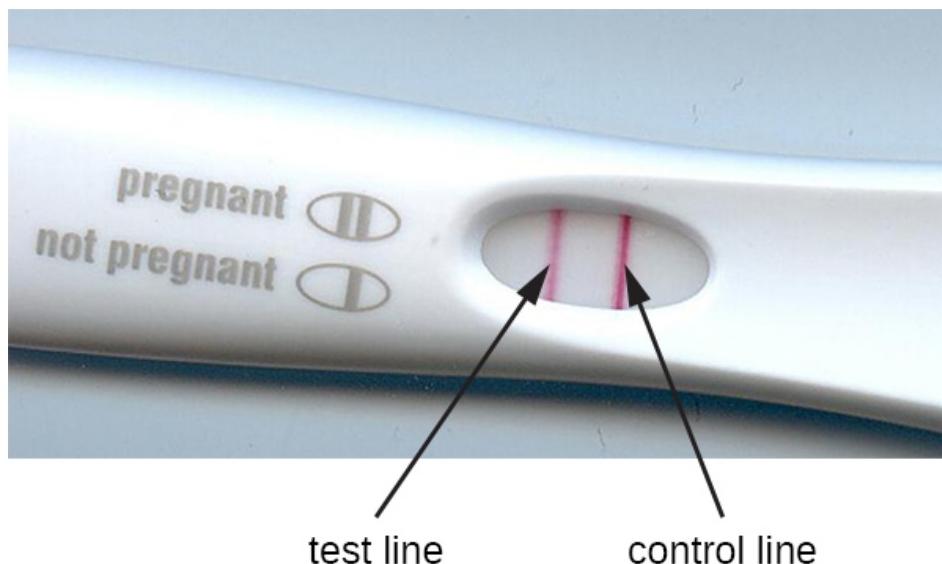
For some situations, it may be necessary to detect or quantify antigens or antibodies that are present at very low concentration in solution. Immunofiltration techniques have been developed to make this possible. In **immunofiltration**, a large volume of fluid is passed through a porous membrane into an absorbent pad. An antigen attached to the porous membrane will capture antibody as it passes; alternatively, we can also attach an antibody to the membrane to capture antigen.

The method of immunofiltration has been adapted in the development of **immunochromatographic assays**, commonly known as **lateral flow tests** or strip tests. These

tests are quick and easy to perform, making them popular for point-of-care use (i.e., in the doctor's office) or in-home use. One example is the TORCH test that allows doctors to screen pregnant women or newborns for infection by an array of viruses and other pathogens (*Toxoplasma*, other viruses, rubella, cytomegalovirus, herpes simplex). In-home pregnancy tests are another widely used example of a lateral flow test ([\[link\]](#)). Immunofiltration tests are also popular in developing countries, because they are inexpensive and do not require constant refrigeration of the dried reagents. However, the technology is also built into some sophisticated laboratory equipment.

In lateral flow tests ([\[link\]](#)), fluids such as urine are applied to an absorbent pad on the test strip. The fluid flows by capillary action and moves through a stripe of beads with antibodies attached to their surfaces. The fluid in the sample actually hydrates the reagents, which are present in a dried state in the stripe. Antibody-coated beads made of latex or tiny gold particles will bind antigens in the test fluid. The antibody-antigen complexes then flow over a second stripe that has immobilized antibody against the antigen; this stripe will retain the beads that have bound antigen. A third control stripe binds any beads. A red color (from gold particles) or blue (from latex beads) developing at the test line indicates a positive test. If the color only develops at the control line, the test is negative.

Like ELISA techniques, lateral flow tests take advantage of antibody sandwiches, providing sensitivity and specificity. While not as quantitative as ELISA, these tests have the advantage of being fast, inexpensive, and not dependent on special equipment. Thus, they can be performed anywhere by anyone. There are some concerns about putting such powerful diagnostic tests into the hands of people who may not understand the tests' limitations, such as the possibility of false-positive results. While home pregnancy tests have become widely accepted, at-home antibody-detection tests for diseases like HIV have raised some concerns in the medical community. Some have questioned whether self-administration of such tests should be allowed in the absence of medical personnel who can explain the test results and order appropriate confirmatory tests. However, with growing numbers of lateral flow tests becoming available, and the rapid development of lab-on-a-chip technology ([\[link\]](#)), home medical tests are likely to become even more commonplace in the future.



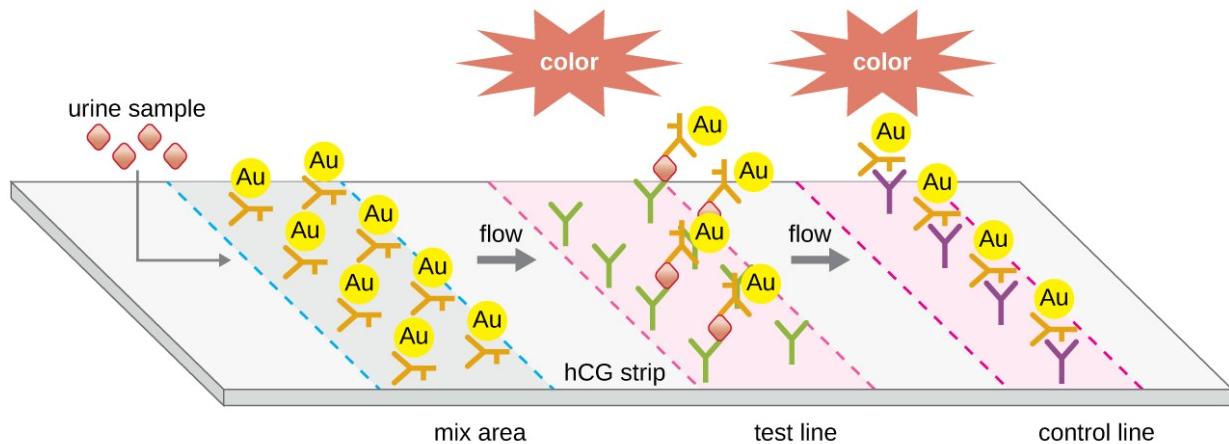
A lateral flow test detecting pregnancy-related hormones in urine.

The control stripe verifies the validity of the test and the test line determines the presence of pregnancy-related hormones in the urine.

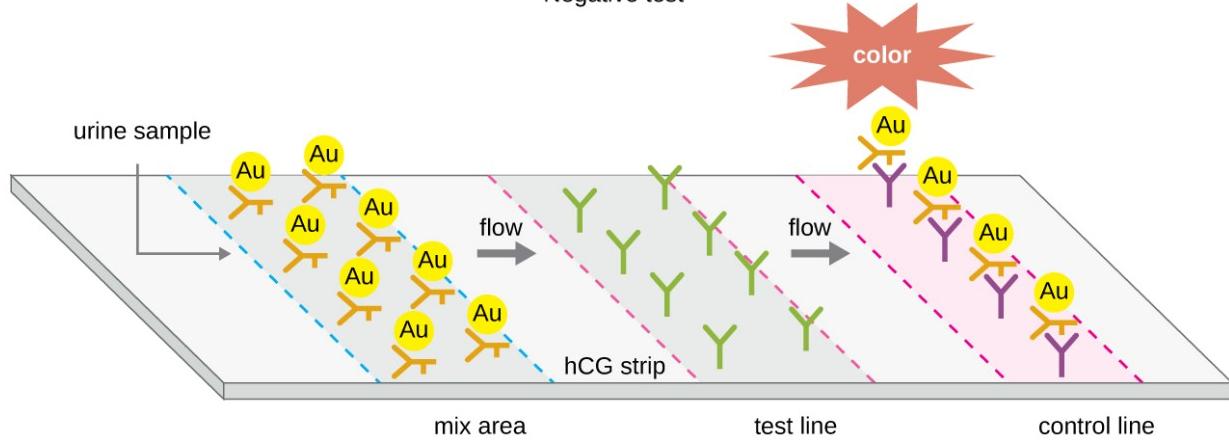
(credit: modification of work by Klaus Hoffmeier)

Positive test

- ① Human chorionic gonadotropin (hCG) urine sample is applied to absorbent sample pad.
- ② hCG antigen bonds with the anti-hCG antibody-colloidal gold conjugates.
- ③ hCG antigen bound to anti-hCG antibody-colloidal gold conjugate is captured by immobilized anti-hCG antibody.
- ④ Free hCG antibody-colloidal gold is captured by antibodies.



Negative test



hCG-first antibody

human chorionic
gonadotropin (hCG)



hCG-second
antibody-AuNPs (hCG-GC)



IgG

Immunochemical assays, or lateral flow tests, allow the testing of antigen in a dilute solution. As the fluid flows through the test strip, it rehydrates the reagents. Antibodies conjugated to small particles bind the antigen in the first stripe and then flow onto the second stripe where they are bound by a second, fixed antibody. This produces a line of color, depending on the color of the beads. The third, control stripe binds beads as well to indicate that the test is working properly. (credit: modification of work by Yeh CH, Zhao ZQ, Shen PL, Lin YC)

Note:

- What physical process does the lateral flow method require to function?
- Explain the purpose of the third strip in a lateral flow assay.

[link] compares some of the key mechanisms and examples of some of the EIAs discussed in this section as well as immunoblots, which were discussed in [Detecting Antigen-Antibody Complexes](#).

Immunoblots & Enzyme Immunoassays			
Type of Assay	Mechanism	Specific Procedures	Examples
Immunoblots	Uses enzyme-antibody conjugates to identify specific proteins that have been transferred to an absorbent membrane	Western blot: Detects the presence of a particular protein	Detecting the presence of HIV peptides (or peptides from other infectious agents) in patient sera
Immunostaining	Uses enzyme-antibody conjugates to stain specific molecules on or in cells	Immunohistochemistry: Used to stain specific cells in a tissue	Stain for presence of CD8 cells in host tissue

Immunoblots & Enzyme Immunoassays			
Type of Assay	Mechanism	Specific Procedures	Examples
Enzyme-linked immunosorbent assay (ELISA)	Uses enzyme-antibody conjugates to quantify target molecules	Direct ELISA: Uses a single antibody to detect the presence of an antigen	Detection of HIV antigen p24 up to one month after being infected
		Indirect ELISA: Measures the amount of antibody produced against an antigen	Detection of HIV antibodies in serum
Immunochromatographic (lateral flow) assays	Techniques use the capture of flowing, color-labeled antigen-antibody complexes by fixed antibody for disease diagnosis	Sandwich ELISA: Measures the amount of antigen bound by the antibody	Detection of antibodies for various pathogens in patient sera (e.g., rapid strep, malaria dipstick)
			Pregnancy test detecting human chorionic gonadotrophin in urine

Note:

Part 3

Although the indirect ELISA for HIV is a sensitive assay, there are several complicating considerations. First, if an infected person is tested too soon after becoming infected, the test can yield false-negative results. The seroconversion window is generally about three weeks, but in some cases, it can be more than two months.

In addition to false negatives, false positives can also occur, usually due to previous infections with other viruses that induce cross-reacting antibodies. The false-positive rate depends on the particular brand of test used, but 0.5% is not unusual.[\[footnote\]](#) Because of the possibility of a false positive, all positive tests are followed up with a confirmatory test. This confirmatory test is often an immunoblot (western blot) in which HIV peptides from the patient's blood are identified using an HIV-specific mAb-enzyme conjugate. A positive western blot would confirm

an HIV infection and a negative blot would confirm the absence of HIV despite the positive ELISA.

Thomas, Justin G., Victor Jaffe, Judith Shaffer, and Jose Abreu, "HIV Testing: US Recommendations 2014," *Osteopathic Family Physician* 6, no. 6 (2014).

Unfortunately, western blots for HIV antigens often yield indeterminant results, in which case, they neither confirm nor invalidate the results of the indirect ELISA. In fact, the rate of indeterminants can be 10–49% (which is why, combined with their cost, western blots are not used for screening). Similar to the indirect ELISA, an indeterminant western blot can occur because of cross-reactivity or previous viral infections, vaccinations, or autoimmune diseases.

- Of the 1300 patients being tested, how many false-positive ELISA tests would be expected?
- Of the false positives, how many indeterminant western blots could be expected?
- How would the hospital address any cases in which a patient's western blot was indeterminant?

Jump to the [previous](#) Clinical Focus box. Jump to the [next](#) Clinical Focus box.

Key Concepts and Summary

- **Enzyme immunoassays (EIA)** are used to visualize and quantify antigens. They use an antibody conjugated to an enzyme to bind the antigen, and the enzyme converts a substrate into an observable end product. The substrate may be either a chromogen or a fluorogen.
- **Immunostaining** is an EIA technique for visualizing cells in a tissue (**immunohistochemistry**) or examining intracellular structures (**immunocytochemistry**).
- **Direct ELISA** is used to quantify an antigen in solution. The primary antibody captures the antigen, and the secondary antibody delivers an enzyme. Production of end product from the chromogenic substrate is directly proportional to the amount of captured antigen.
- **Indirect ELISA** is used to detect antibodies in patient serum by attaching antigen to the well of a microtiter plate, allowing the patient (primary) antibody to bind the antigen and an enzyme-conjugated secondary antibody to detect the primary antibody.
- **Immunofiltration and immunochromatographic assays** are used in **lateral flow tests**, which can be used to diagnose pregnancy and various diseases by detecting color-labeled antigen-antibody complexes in urine or other fluid samples

Multiple Choice

Exercise:

Problem: In an enzyme immunoassay, the enzyme

- a. is bound by the antibody's antigen-binding site.
- b. is attached to the well of a microtiter plate.
- c. is conjugated to the suspect antigen.
- d. is bound to the constant region of the secondary antibody.

Solution:

D

Exercise:**Problem:**

When using an EIA to study microtubules or other structures inside a cell, we first chemically fix the cell and then treat the cells with alcohol. What is the purpose of this alcohol treatment?

- a. It makes holes in the cell membrane large enough for antibodies to pass.
 - b. It makes the membrane sticky so antibodies will bind and be taken up by receptor-mediated endocytosis.
 - c. It removes negative charges from the membrane, which would otherwise repulse the antibodies.
 - d. It prevents nonspecific binding of the antibodies to the cell membrane.
-

Solution:

A

Exercise:**Problem:**

In a lateral-flow pregnancy test, you see a blue band form on the control line and no band form on the test line. This is probably a _____ test for pregnancy.

- a. positive
 - b. false-positive
 - c. false-negative
 - d. negative
-

Solution:

D

Exercise:**Problem:**

When performing an FEIA, the fluorogen replaces the _____ that is used in an EIA.

- a. antigen
- b. chromogenic substrate
- c. enzyme
- d. secondary antibody

Solution:

B

Fill in the Blank**Exercise:****Problem:**

To detect antibodies against bacteria in the bloodstream using an EIA, we would run a(n) _____, which we would start by attaching antigen from the bacteria to the wells of a microtiter plate.

Solution:

indirect ELISA

Short Answer**Exercise:****Problem:**

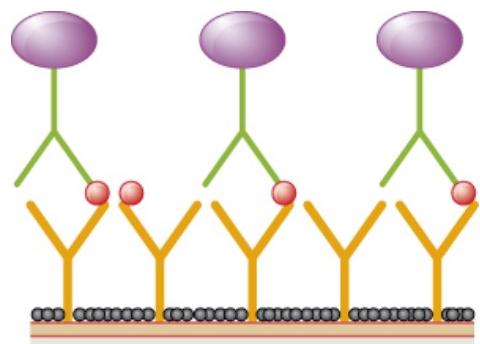
Why is it important in a sandwich ELISA that the antigen has multiple epitopes? And why might it be advantageous to use polyclonal antisera rather than mAb in this assay?

Exercise:**Problem:**

The pregnancy test strip detects the presence of human chorionic gonadotrophin in urine. This hormone is initially produced by the fetus and later by the placenta. Why is the test strip preferred for this test rather than using either a direct or indirect ELISA with their more quantifiable results?

Critical Thinking**Exercise:****Problem:**

Label the primary and secondary antibodies, and discuss why the production of end product will be proportional to the amount of antigen.



Fluorescent Antibody Techniques

LEARNING OBJECTIVES

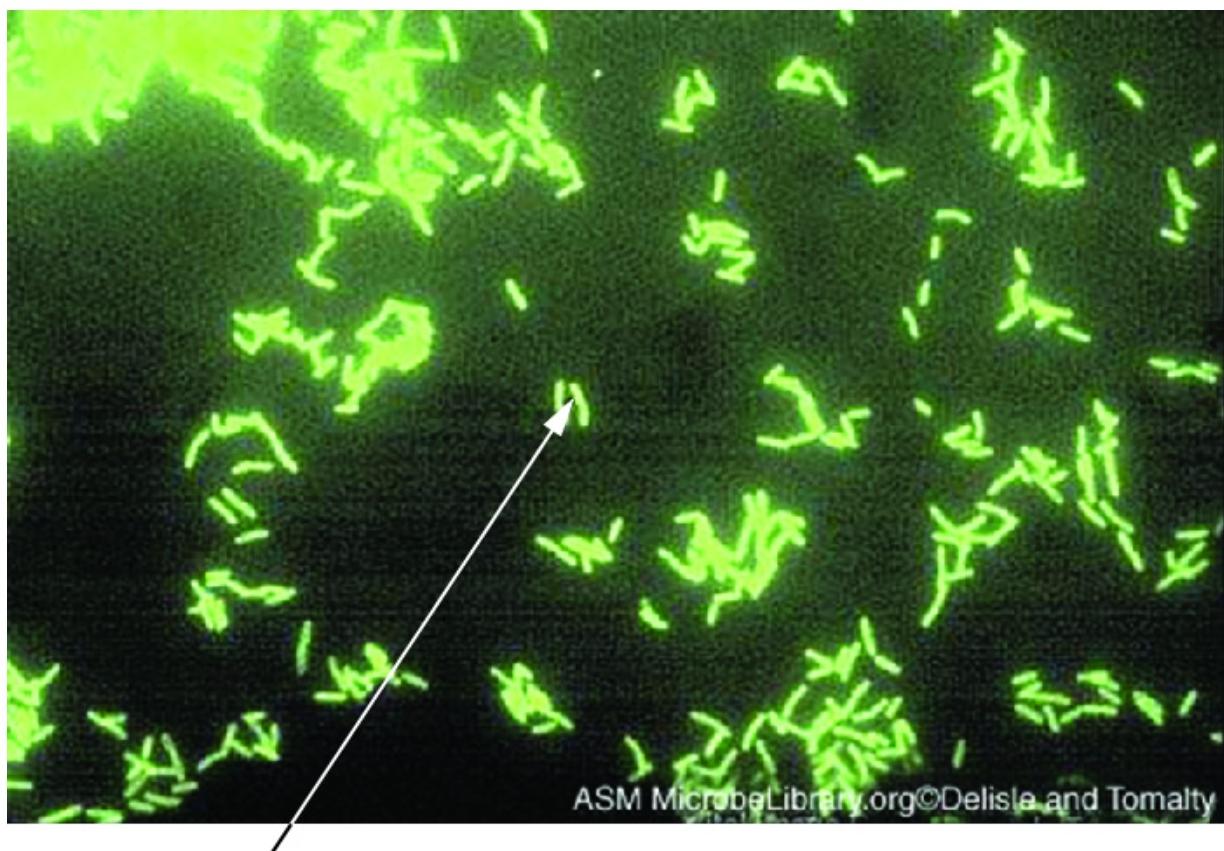
- Describe the benefits of immunofluorescent antibody assays in comparison to nonfluorescent assays
- Compare direct and indirect fluorescent antibody assays
- Explain how a flow cytometer can be used to quantify specific subsets of cells present in a complex mixture of cell types
- Explain how a fluorescence-activated cell sorter can be used to separate unique types of cells

Rapid visualization of bacteria from a clinical sample such as a throat swab or sputum can be achieved through **fluorescent antibody (FA) techniques** that attach a fluorescent marker (fluorogen) to the constant region of an antibody, resulting in a reporter molecule that is quick to use, easy to see or measure, and able to bind to target markers with high specificity. We can also label cells, allowing us to precisely quantify particular subsets of cells or even purify these subsets for further research.

As with the enzyme assays, FA methods may be direct, in which a labeled mAb binds an antigen, or indirect, in which secondary polyclonal antibodies bind patient antibodies that react to a prepared antigen. Applications of these two methods were demonstrated in [\[link\]](#). FA methods are also used in automated cell counting and sorting systems to enumerate or segregate labeled subpopulations of cells in a sample.

Direct Fluorescent Antibody Techniques

Direct fluorescent antibody (DFA) tests use a fluorescently labeled mAb to bind and illuminate a target antigen. DFA tests are particularly useful for the rapid diagnosis of bacterial diseases. For example, fluorescence-labeled antibodies against *Streptococcus pyogenes* (group A strep) can be used to obtain a diagnosis of strep throat from a throat swab. The diagnosis is ready in a matter of minutes, and the patient can be started on antibiotics before even leaving the clinic. DFA techniques may also be used to diagnose pneumonia caused by *Mycoplasma pneumoniae* or *Legionella pneumophila* from sputum samples ([\[link\]](#)). The fluorescent antibodies bind to the bacteria on a microscope slide, allowing ready detection of the bacteria using a fluorescence microscope. Thus, the DFA technique is valuable for visualizing certain bacteria that are difficult to isolate or culture from patient samples.



Fluorescein-labeled antibody attached to *Legionella* bacilli

A green fluorescent mAb against *L. pneumophila* is used here to

visualize and identify bacteria from a smear of a sample from the respiratory tract of a pneumonia patient. (credit: modification of work by American Society for Microbiology)

Note:



Watch the [animation](#) on this page to review the procedures of the direct fluorescent antibody test.

Note:

- In a direct fluorescent antibody test, what does the fluorescent antibody bind to?

Indirect Fluorescent Antibody Techniques

Indirect fluorescent antibody (IFA) tests ([\[link\]](#)) are used to look for antibodies in patient serum. For example, an IFA test for the diagnosis of syphilis uses *T. pallidum* cells isolated from a lab animal (the bacteria cannot be grown on lab media) and a smear prepared on a glass slide. Patient serum is spread over the smear and anti-treponemal antibodies, if

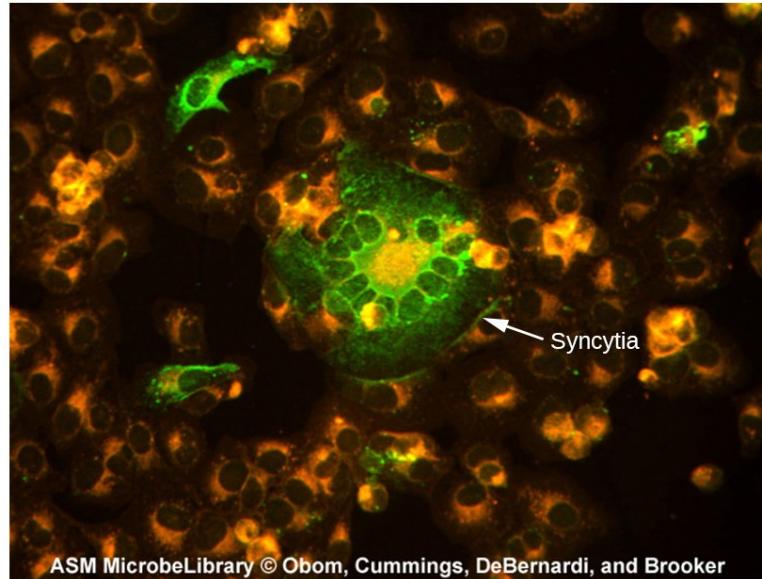
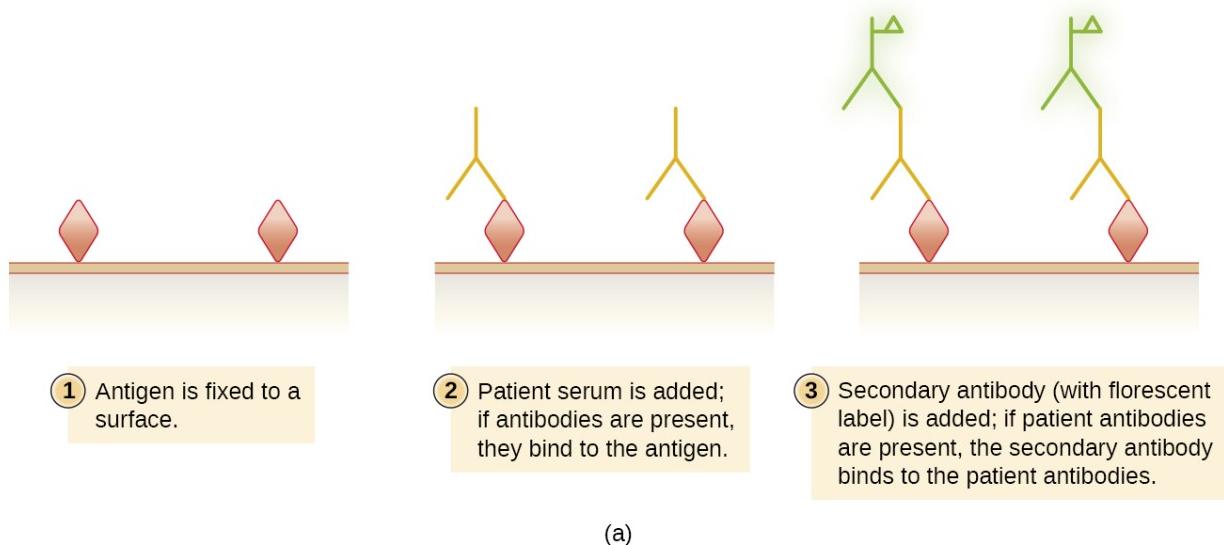
present, are allowed to bind. The serum is washed off and a secondary antibody added. The secondary antibody is an antihuman immunoglobulin conjugated to a fluorogen. On examination, the *T. pallidum* bacteria will only be visible if they have been bound by the antibodies from the patient's serum.

The IFA test for syphilis provides an important complement to the VDRL test discussed in [Detecting Antigen-Antibody Complexes](#). The VDRL is more likely to generate false-positive reactions than the IFA test; however, the VDRL is a better test for determining whether an infection is currently active.

IFA tests are also useful for the diagnosis of autoimmune diseases. For example, systemic lupus erythematosus (SLE) (see [Autoimmune Disorders](#)) is characterized by elevated expression levels of antinuclear antibodies (ANA). These autoantibodies can be expressed against a variety of DNA-binding proteins and even against DNA itself. Because autoimmunity is often difficult to diagnose, especially early in disease progression, testing for ANA can be a valuable clue in making a diagnosis and starting appropriate treatment.

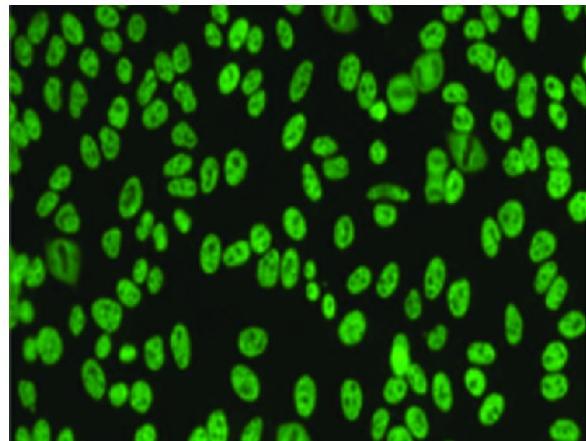
The IFA for ANA begins by fixing cells grown in culture to a glass slide and making them permeable to antibody. The slides are then incubated with serial dilutions of serum from the patient. After incubation, the slide is washed to remove unbound proteins, and the fluorescent antibody (antihuman IgG conjugated to a fluorogen) added. After an incubation and wash, the cells can be examined for fluorescence evident around the nucleus ([\[link\]](#)). The titer of ANA in the serum is determined by the highest dilution showing fluorescence. Because many healthy people express ANA, the American College of Rheumatology recommends that the titer must be at least 1:40 in the presence of symptoms involving two or more organ systems to be considered indicative of SLE.[\[footnote\]](#)

Gill, James M., ANNA M. Quisel, PETER V. Rocca, and DENE T. Walters. "Diagnosis of systemic lupus erythematosus." *American family physician* 68, no. 11 (2003): 2179-2186.

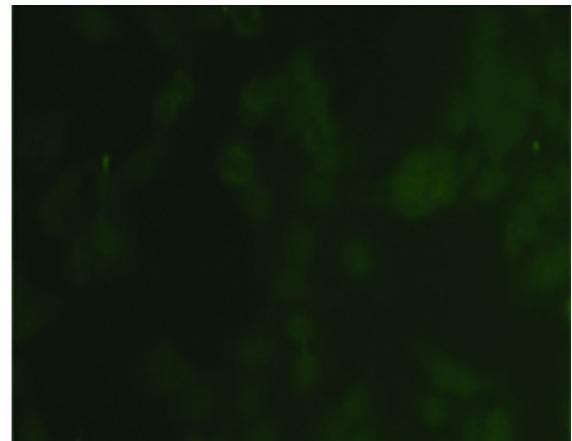


(b)

(a) The IFA test is used to detect antigen-specific antibodies by allowing them to bind to antigen fixed to a surface and then illuminating these complexes with a secondary antibody-fluorogen conjugate. (b) In this example of a micrograph of an indirect fluorescent antibody test, a patient's antibodies to the measles virus bind to viral antigens present on inactivated measles-infected cells affixed to a slide. Secondary antibodies bind the patient's antibodies and carry a fluorescent molecule. (credit b: modification of work by American Society for Microbiology)



diseased



healthy control

In this test for antinuclear antibodies (ANA), cells are exposed to serum from a patient suspected of making ANA and then to a fluorescent mAb specific for human immunoglobulin. As a control, serum from a healthy patient is also used. Visible fluorescence around the nucleus demonstrates the presence of ANA in the patient's serum. In the healthy control where lower levels of ANA are produced, very faint green is detected. (credit left, right: modification of work by Al-Hussaini AA, Alzahrani MD, Alenizi AS, Suliman NM, Khan MA, Alharbi SA, Chentoufi AA)

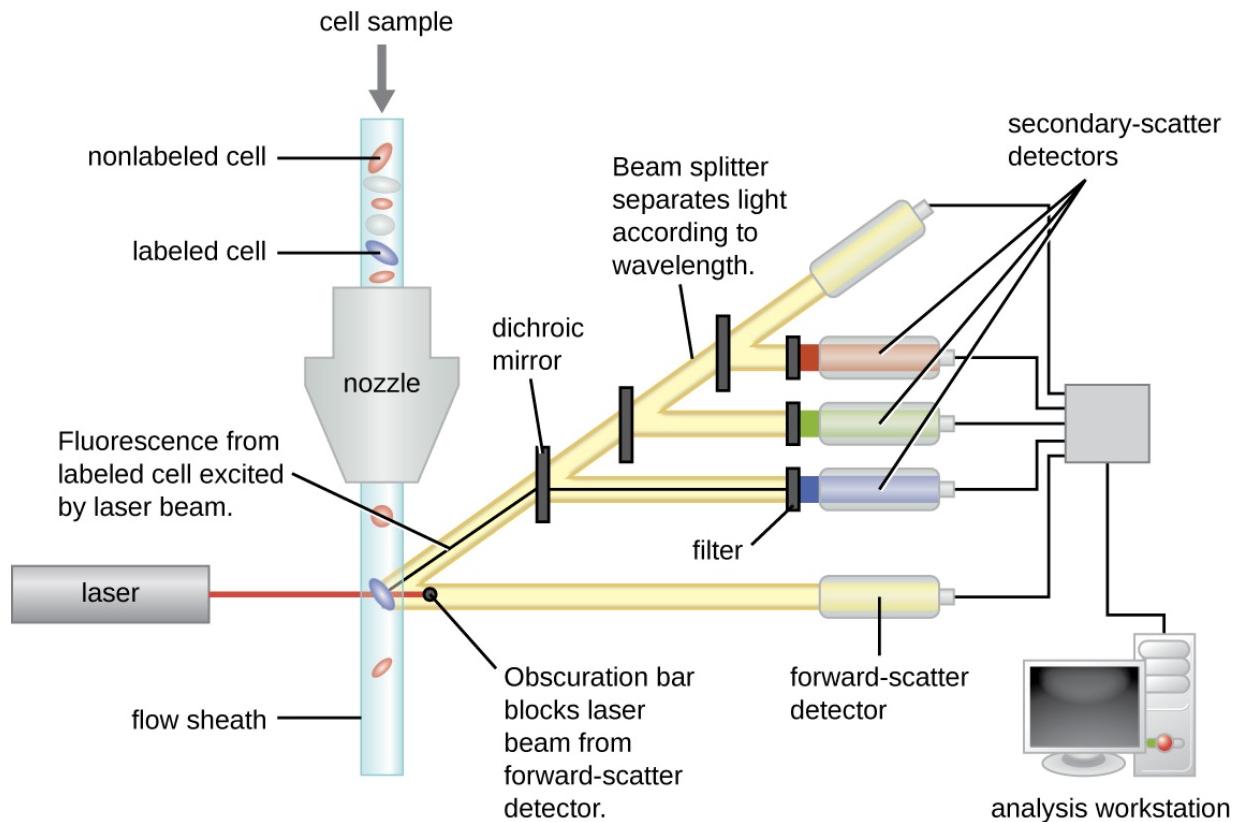
Note:

- In an indirect fluorescent antibody test, what does the fluorescent antibody bind to?
- What is the ANA test looking for?

Flow Cytometry

Fluorescently labeled antibodies can be used to quantify cells of a specific type in a complex mixture using **flow cytometry** ([\[link\]](#)), an automated, cell-counting system that detects fluorescing cells as they pass through a narrow tube one cell at a time. For example, in HIV infections, it is important to know the level of CD4 T cells in the patient's blood; if the numbers fall below 500 per μL of blood, the patient becomes more likely to acquire opportunistic infections; below 200 per μL , the patient can no longer mount a useful adaptive immune response at all. The analysis begins by incubating a mixed-cell population (e.g., white blood cells from a donor) with a fluorescently labeled mAb specific for a subpopulation of cells (e.g., anti-CD4). Some experiments look at two cell markers simultaneously by adding a different fluorogen to the appropriate mAb. The cells are then introduced to the flow cytometer through a narrow capillary that forces the cells to pass in single file. A laser is used to activate the fluorogen. The fluorescent light radiates out in all directions, so the fluorescence detector can be positioned at an angle from the incident laser light.

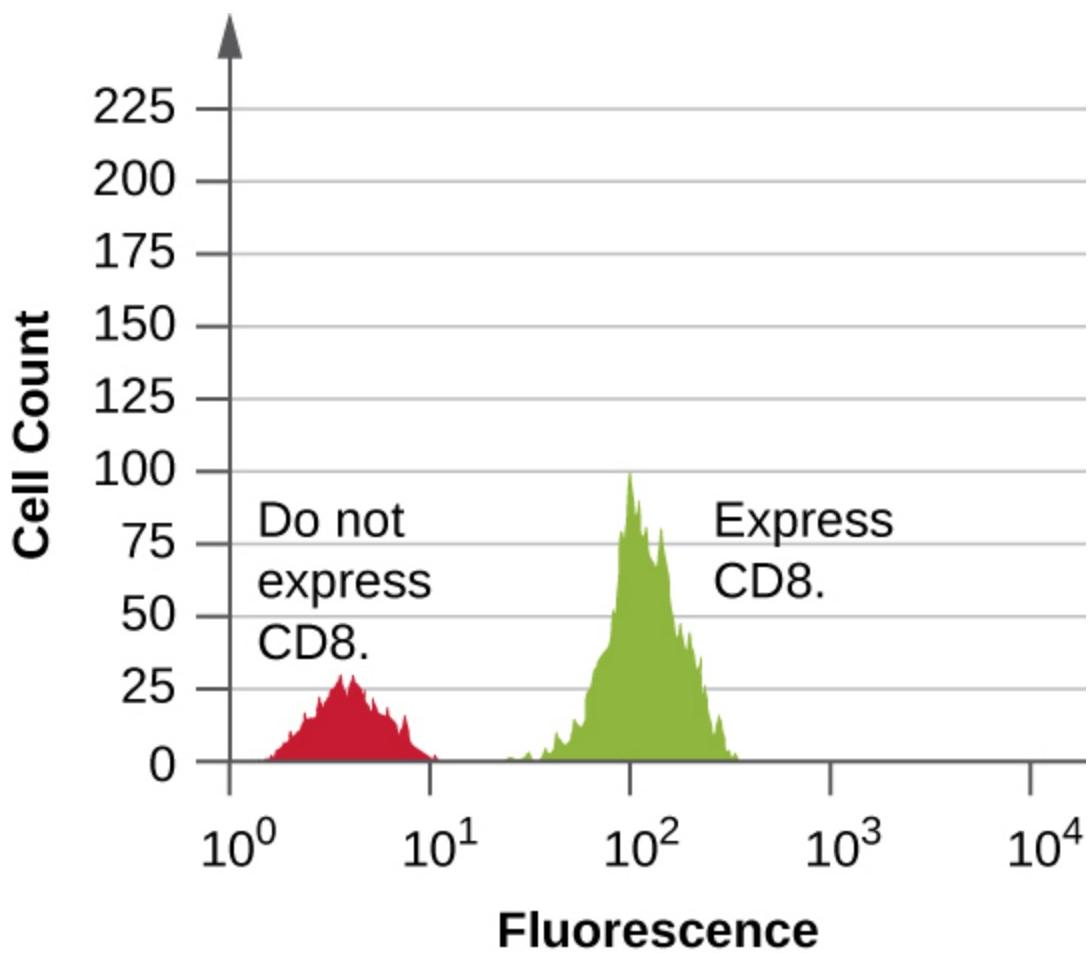
[\[link\]](#) shows the obscuration bar in front of the forward-scatter detector that prevents laser light from hitting the detector. As a cell passes through the laser bar, the forward-scatter detector detects light scattered around the obscuration bar. The scattered light is transformed into a voltage pulse, and the cytometer counts a cell. The fluorescence from a labeled cell is detected by the side-scatter detectors. The light passes through various dichroic mirrors such that the light emitted from the fluorophore is received by the correct detector.



In flow cytometry, a mixture of fluorescently labeled and unlabeled cells passes through a narrow capillary. A laser excites the fluorogen, and the fluorescence intensity of each cell is measured by a detector.
 (credit: modification of work by “Kierano”/Wikimedia Commons)

Data are collected from both the forward- and side-scatter detectors. One way these data can be presented is in the form of a histogram. The forward scatter is placed on the y-axis (to represent the number of cells), and the side scatter is placed on the x-axis (to represent the fluorescence of each cell). The scaling for the x-axis is logarithmic, so fluorescence intensity increases by a factor of 10 with each unit increase along the axis. [\[link\]](#) depicts an example in which a culture of cells is combined with an antibody attached to a fluorophore to detect CD8 cells and then analyzed by flow cytometry. The histogram has two peaks. The peak on the left has lower fluorescence readings, representing the subset of the cell population (approximately 30 cells) that does not fluoresce; hence, they are not bound

by antibody and therefore do not express CD8. The peak on the right has higher fluorescence readings, representing the subset of the cell population (approximately 100 cells) that show fluorescence; hence, they are bound by the antibody and therefore do express CD8.



Flow cytometry data are often compiled as a histogram. In the histogram, the area under each peak is proportional to the number of cells in each population. The x-axis is the relative fluorescence expressed by the cells (on a log scale), and the y-axis represents the number of cells at a particular level of fluorescence.

Note:

- What is the purpose of the laser in a flow cytometer?
- In the output from a flow cytometer, the area under the histogram is equivalent to what?

Note:**Resolution**

After notifying all 1300 patients, the hospital begins scheduling HIV screening. Appointments were scheduled a minimum of 3 weeks after the patient's last hospital visit to minimize the risk of false negatives. Because some false positives were anticipated, the public health physician set up a counseling protocol for any patient whose indirect ELISA came back positive.

Of the 1300 patients, eight tested positive using the ELISA. Five of these tests were invalidated by negative western blot tests, but one western blot came back positive, confirming that the patient had indeed contracted HIV. The two remaining western blots came back indeterminate. These individuals had to submit to a third test, a PCR, to confirm the presence or absence of HIV sequences. Luckily, both patients tested negative.

As for the lone patient confirmed to have HIV, the tests cannot prove or disprove any connection to the syringes compromised by the former hospital employee. Even so, the hospital's insurance will fully cover the patient's treatment, which began immediately.

Although we now have drugs that are typically effective at controlling the progression of HIV and AIDS, there is still no cure. If left untreated, or if the drug regimen fails, the patient will experience a gradual decline in the number of CD4 helper T cells, resulting in severe impairment of all adaptive immune functions. Even moderate declines of helper T cell numbers can result in immunodeficiency, leaving the patient susceptible to opportunistic infections. To monitor the status of the patient's helper T cells, the hospital will use flow cytometry. This sensitive test allows physicians to precisely determine the number of helper T cells so they can adjust treatment if the number falls below 500 cells/ μL .

Jump to the [previous](#) Clinical Focus box.

Cell Sorting Using Immunofluorescence

The flow cytometer and immunofluorescence can also be modified to sort cells from a single sample into purified subpopulations of cells for research purposes. This modification of the flow cytometer is called a **fluorescence-activated cell sorter (FACS)**. In a FACS, fluorescence by a cell induces the device to put a charge on a droplet of the transporting fluid containing that cell. The charge is specific to the wavelength of the fluorescent light, which allows for differential sorting by those different charges. The sorting is accomplished by an electrostatic deflector that moves the charged droplet containing the cell into one collecting vessel or another. The process results in highly purified subpopulations of cells.

One limitation of a FACS is that it only works on isolated cells. Thus, the method would work in sorting white blood cells, since they exist as isolated cells. But for cells in a tissue, flow cytometry can only be applied if we can excise the tissue and separate it into single cells (using proteases to cleave cell-cell adhesion molecules) without disrupting cell integrity. This method may be used on tumors, but more often, immunohistochemistry and immunocytochemistry are used to study cells in tissues.

Note:



Watch videos to learn more about how [flow cytometry](#) and a [FACS](#) work.

Note:

- In fluorescence activated cell sorting, what characteristic of the target cells allows them to be separated?

[[link](#)] compares the mechanisms of the fluorescent antibody techniques discussed in this section.

Fluorescent Antibody Techniques		
Type of Assay	Mechanism	Examples
Direct fluorescent antibody (DFA)	Uses fluorogen-antibody conjugates to label bacteria from patient samples	Visualizing <i>Legionella pneumophila</i> from a throat swab
Indirect fluorescent antibody (IFA)	Detects disease-specific antibodies in patient serum	Diagnosing syphilis; detecting antinuclear antibodies (ANA) for lupus and other autoimmune diseases

Fluorescent Antibody Techniques

Type of Assay	Mechanism	Examples
Flow cytometry	Labels cell membranes with fluorogen-antibody conjugate markers excited by a laser; machine counts the cell and records the relative fluorescence	Counting the number of fluorescently labeled CD4 or CD8 cells in a sample
Fluorescence activated cell sorter (FACS)	Form of flow cytometry that both counts cells and physically separates them into pools of high and low fluorescence cells	Sorting cancer cells

Key Concepts and Summary

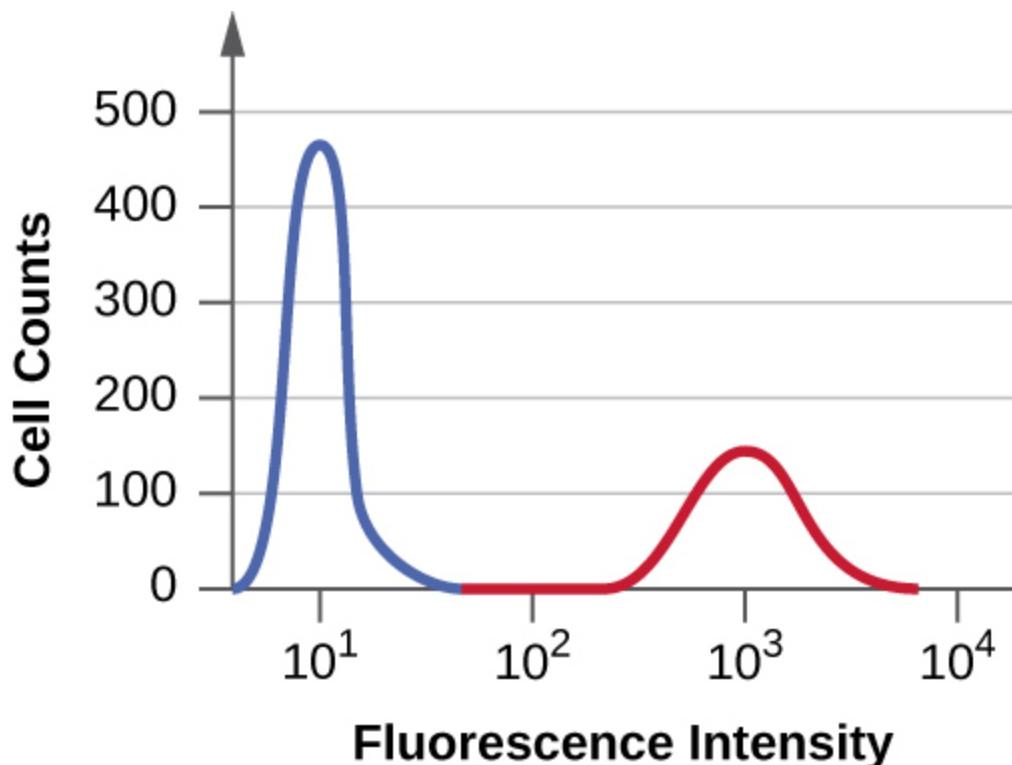
- **Immunofluorescence** assays use antibody-fluorogen conjugates to illuminate antigens for easy, rapid detection.
- **Direct immunofluorescence** can be used to detect the presence of bacteria in clinical samples such as sputum.
- **Indirect immunofluorescence** detects the presence of antigen-specific antibodies in patient sera. The fluorescent antibody binds to the antigen-specific antibody rather than the antigen.
- The use of indirect immunofluorescence assays to detect **antinuclear antibodies** is an important tool in the diagnosis of several autoimmune diseases.
- **Flow cytometry** uses fluorescent mAbs against cell-membrane proteins to quantify specific subsets of cells in complex mixtures.
- **Fluorescence-activated cell sorters** are an extension of flow cytometry in which fluorescence intensity is used to physically separate cells into high and low fluorescence populations.

Multiple Choice

Exercise:

Problem:

Suppose you need to quantify the level of CD8 T cells in the blood of a patient recovering from influenza. You treat a sample of the patient's white blood cells using a fluorescent mAb against CD8, pass the cells through a flow cytometer, and produce the histogram shown below. The area under the peak to the left (blue) is three times greater than the area of the peak on the right (red). What can you determine from these data?



- a. There are no detectable CD8 cells.
- b. There are three times as many CD4 cells than CD8 cells.
- c. There are three times as many CD8 cells than CD4 cells.
- d. CD8 cells make up about one-fourth of the total number of cells.

Solution:

D

Exercise:

Problem:

In the data described in the previous question, the average fluorescence intensity of cells in the second (red) peak is about _____ that in the first (blue) peak.

- a. three times
 - b. 100 times
 - c. one-third
 - d. 1000 times
-

Solution:

B

Exercise:

Problem:

In a direct fluorescent antibody test, which of the following would we most likely be looking for using a fluorescently-labeled mAb?

- a. bacteria in a patient sample
 - b. bacteria isolated from a patient and grown on agar plates
 - c. antiserum from a patient smeared onto a glass slide
 - d. antiserum from a patient that had bound to antigen-coated beads
-

Solution:

A

Fill in the Blank

Exercise:

Problem:

In flow cytometry, cell subsets are labeled using a fluorescent antibody to a membrane protein. The fluorogen is activated by a(n) _____ as the cells pass by the detectors.

Solution:

laser

Exercise:**Problem:**

Fluorescence in a flow cytometer is measured by a detector set at an angle to the light source. There is also an in-line detector that can detect cell clumps or _____.

Solution:

fragments

Critical Thinking

Exercise:**Problem:**

A patient suspected of having syphilis is tested using both the VDRL test and IFA. The IFA test comes back positive, but the VDRL test is negative. What is the most likely reason for these results?

Exercise:

Problem:

A clinician suspects that a patient with pneumonia may be infected by *Legionella pneumophila*. Briefly describe two reasons why a DFA test might be better for detecting this pathogen than standard bacteriology techniques.

Under Construction

Beer & Wine Production

Beer

Beer making is combination of science and art. Beer requires only 4 ingredients in its simplest form: barley, water, hops and yeast. It relies on enzymes provided by both the barley and yeast for the conversion from complex plant starches to alcohol. Hops mainly play a flavoring role along with other purposes. There is an art to how these components are brought together. If you have ever tasted a friend's home brew, you understand the difference of science going right versus it going very wrong.

When done correctly, beer making can take on great levels of complexity depending on the brewer; however, the basic process is shown in Figure 1. This process can be thought of in the following steps:



Figure 1. The beer making process (By J.P.Lon at English Wikipedia, CC BY-SA 3.0, <https://commons.wikimedia.org/w/index.php?curid=7161486>)

1. **Malting.** Malting the first step, is the pivotal process of preparing barley, a grain, for the next steps. Barley contains starch, which is insoluble in water and inaccessible to yeasts, at the beginning in the kernel. Each step of the malting process helps break the starches as well as other nutrients of the

kernel (protein, fats) down. Steeping is the first step in malting, where the grain is steeped (soaked) in water for about 40 hours. The next step is germination of the barley grain. To germinate the barley, the grain is spread out on the floor of the germination room where rootlets begin to form. Upon germination, the barley seed starts to activate its own enzymes to break down the starch into sugars and its proteins into amino acids. The barley plant wants to use that sugar, for instance, as energy to grow into a sprout. The germination process produces the enzymes which break down the starches within the grain into shorter length polysaccharides. At the end of the germination process, about three days, the starch has become partially converted to short chain sugars but not completely. The barley grain is called green malt at this point and has developed small roots (Figure 2).



Figure 2. Green malt before kilning

2. Kilning. The germination is halted by drying the green malt on metal racks in the kiln house. The temperature is then raised to 85°C for a light malt or higher for a dark malt. It is important that temperature increases are gradual so that the enzymes in the grain are not denatured but the sprouting is stopped. The malt roots are removed, and the dried malt (which look like seeds) is stored. Although malted barley is the primary ingredient, unmalted corn, rice or wheat (termed adjuncts) are sometimes added, to impart different beer flavors. The differences in the way the barley is kilned will affect the flavor, color and aroma of the beer. If you kiln the barley longer

and at higher temperatures, the darker and more chocolate-flavored the beer will be. The strongly roasted malt will be used for stout beer while less roasted malt, called pale malt, can be used in lighter colored beers like pilsners.

3. Mashing. Milling is the cracking of the grain before mashing. Milling the grain allows it to absorb the water it will eventually be mixed with in order for the water to extract sugars from the malt. Mashing is the process of turning the finely-ground malt, called the grist, into a sweetened liquid called wort. During mashing, the milled grain is added to warm water in a large cooking vessel called the mash tun (Figure 3). In this mash tun, the grain and heated water mix creating a cereal-like mash. The barley enzymes finish what they started in malting: they convert the starches and other shorter polysaccharides, which were released during the malting stage, to simple sugars that can be fermented. Other conversions, such as proteins to amino acids, also happen. This sugar rich liquid is then strained through the bottom of the mash (a process called lautering) and is now called wort.



Figure 3. A large mash tun with a rake implement for mixing the mash (<https://commons.wikimedia.org/w/index.php?curid=97642>)

4. Boiling/hopping. As the wort is heated, certain types of hops, which are flowers of the plant *Humulus lupulus*, are added at different times during

the boil for either bitterness or aroma and to help preserve it. The wort is boiled for one to two hours to concentrate it as well as extract the necessary essence from the hops. Hops also release compounds that inhibit the growth of bacteria that would spoil the beer later.

5. Cooling. The wort is transferred quickly from the brew kettle through a device to filter out the hops and then onto a heat exchanger to be cooled. It is important to quickly cool the wort to a point where yeast can safely be added because yeast does not grow in high heat. Cooling also causes hop solids, known as the trub, to precipitate out of the wort. The hopped wort is then saturated with air, essential for the growth of the yeast in the next stage. At this point, the wort can be considered a growth medium prepared for the yeast.

6. Fermentation. The cooled wort goes to the fermentation tank where the brewer adds a type of yeast. The yeasts ferment the sugar in the wort and turns it into alcohol and carbon dioxide via alcoholic fermentation (Figure 4). This process of fermentation takes about ten days. Each brewery has its own strains of yeast, which largely determine the character of the beer. Most beers are either ales or lagers. With ale yeast varieties (*Saccharomyces cerevisiae*), the cells ferment at higher temperatures and rise to the top at the end of fermentation. On the other hand, lager yeast strains (*Saccharomyces carlsbergensis*) ferment at lower temperatures and sink to the bottom. Ales are generally more fruity flavored due to greater production of esters by the ale yeast at higher temperatures while lagers are more malty tasting because of a lower production rate of esters by the lager yeasts at the lower temperatures.



Figure 4. An open style fermenter containing fermenting wort covered with foam. (<https://commons.wikimedia.org/w/index.php?curid=39527655>)

7. Maturation (also called racking). The beer has now been brewed, but it can still be improved through maturation. During this phase, the brewer moves, or racks, the beer into a new tank called the conditioning tank, which helps remove sediment. The brewer then waits for the beer to complete its aging process. The taste ripens as the liquid is clarified. The brewer tries to prevent oxygen from entering the beer so that contamination microbes cannot grow in it. The microbes would contribute lactic acid and other off-flavors to the beer.

8. Finishing. Here the beer is pasteurized and/or filtered to give the beer clarity. The beer is moved to a holding tank where it stays until it is bottled, canned or put into kegs. Finally it can be enjoyed!

Cheese Production

Cheese making

For a true microbiologist, the process of cheese making may be the most amazing phenomenon ever witnessed; solar eclipses and childbirth pale in comparison. The major event in this process is the conversion of liquid milk to a solid consisting mainly of casein protein and fat, which is the foundation of young cheese. The key step in this conversion is the denaturation of casein, which changes it from a dissolved protein in milk to an insoluble component of a solid (curd) that separates from the watery portion of the milk (whey). Two factors help trigger this denaturation. First of all, good bacteria or “starter cultures” are added to the milk to ferment the lactose, milk’s natural sugar, into lactic acid, which causes a pH change to help start denaturation of casein. Different types of starter cultures also produce other metabolites besides lactic acid, which create different types of cheese flavors. For example, Swiss cheese uses one type of culture, while brie and blue cheese use others. Second, rennin, an enzyme originally isolated from calf stomachs, also help denature casein by proteolyzing it. Upon denaturation, the casein unfolds and then aggregates with fat in the milk, forming the curd, which separates from the liquid whey (Figure 1). This process is known as coagulation or curdling. Whey contains mainly water, salts and other milk proteins.



Figure 1. White curd has separated from the yellow whey after curdling.

Once the curd starts to gel, the cheesemakers cut it up, which allows the whey to come out (Figure 2). Drier cheeses are often cut more to form smaller curds, so more of the moisture comes out. Once the curds are cut, they are stirred and heated to release even more whey and further denature the casein. At this point, the curd is separated from the whey and is salted. Then the cheese is pressed and eventually becomes the shape and consistency of recognizable cheeses.



Figure 2. Curd being broken up by mixers in industrial scale production.
(<https://commons.wikimedia.org/w/index.php?curid=246468>)

Once the cheese is shaped, it may be aged a while, allowing the bacteria and/or fungi in the starter cultures to produce different fermentation products and other flavorings for the cheese (termed ripening). For instance, for Swiss cheese, *Propionibacterium* create propionic acid via fermentation to give it a nutty flavor. Also, other types of microbes can be added at this later time to the young cheeses to help produce more flavors. Some cheeses are aged for years before serving. However, a small taste of a properly aged Parmesan or Gorgonzola will justify the wait and further help you

appreciate the splendor of this process! The major steps in this process are summarized in Figure 3.

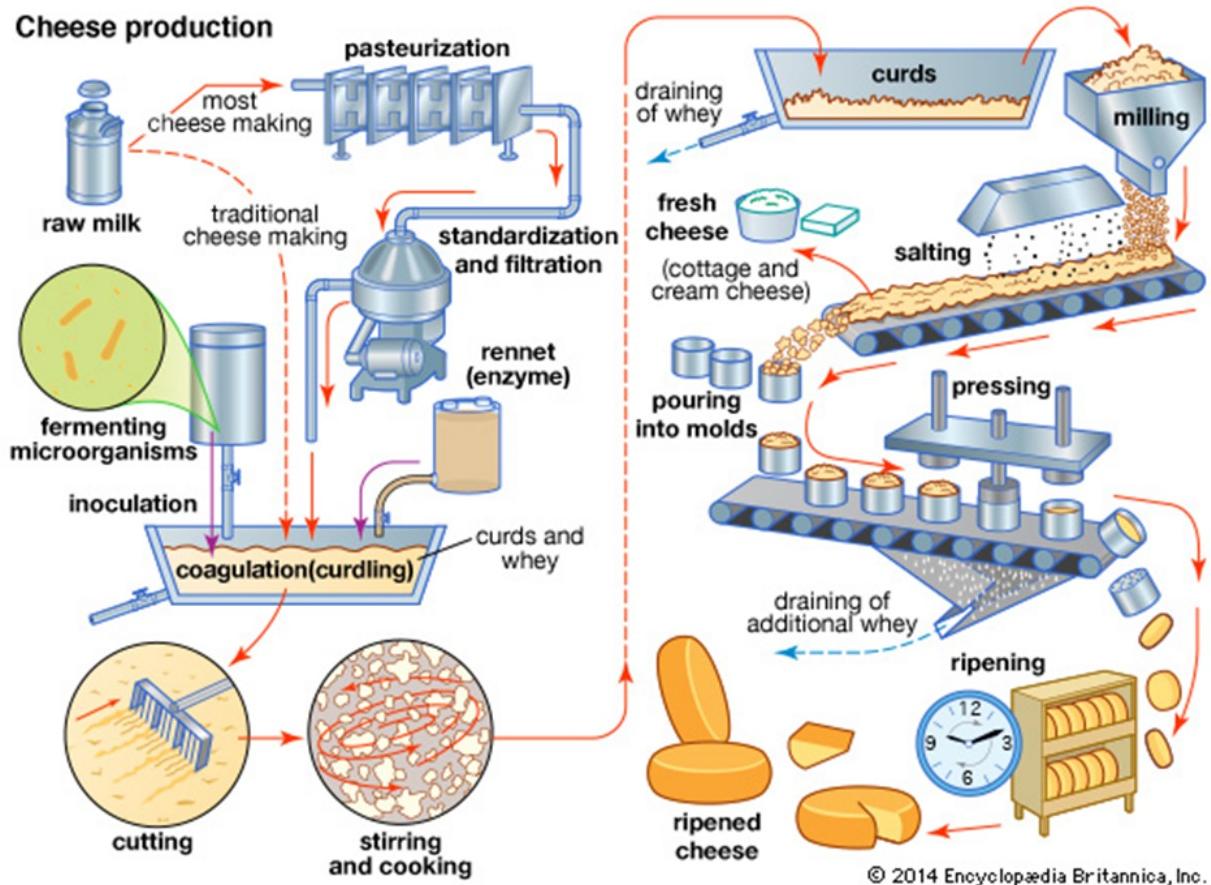


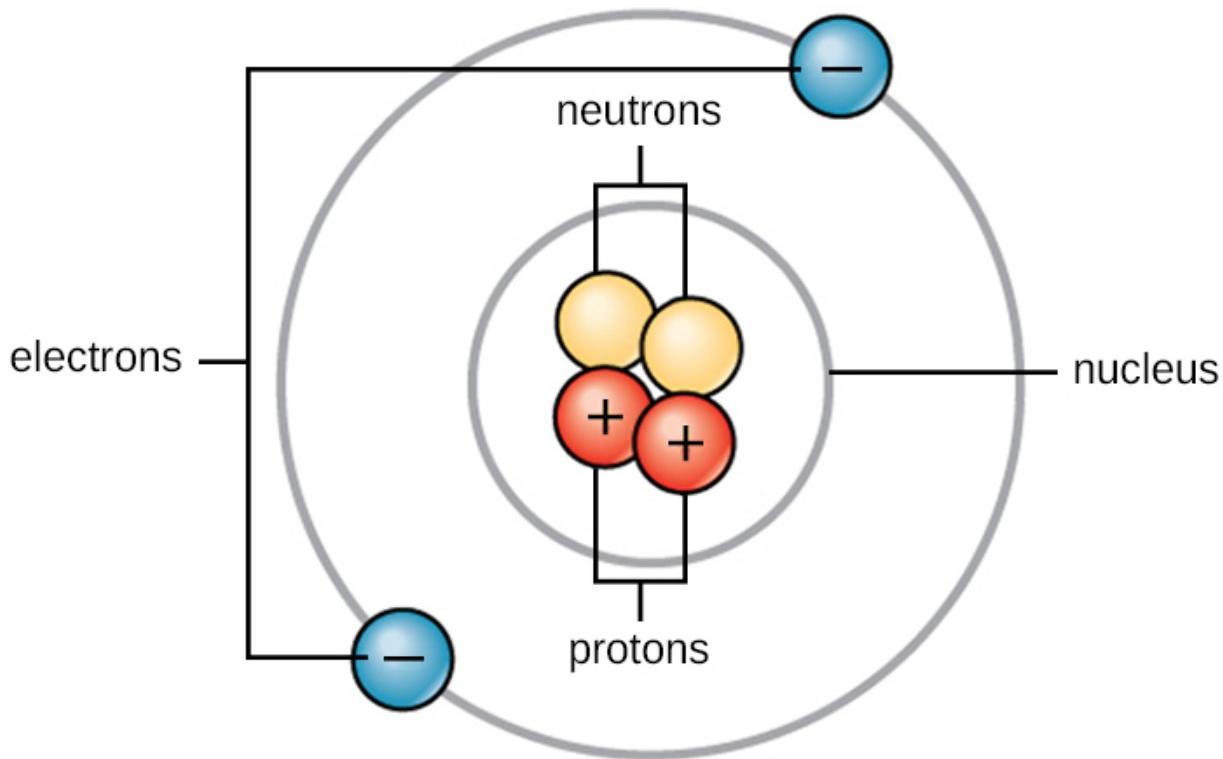
Figure 3. The basic steps in the cheese making process
(www.Encyclopaedia Britannica.com)

Fundamentals of Physics and Chemistry Important to Microbiology

Like all other matter, the matter that comprises microorganisms is governed by the laws of chemistry and physics. The chemical and physical properties of microbial pathogens—both cellular and acellular—dictate their habitat, control their metabolic processes, and determine how they interact with the human body. This appendix provides a review of some of the fundamental principles of chemistry and physics that are essential to an understanding of microbiology. Many of the chapters in this text—especially [Microbial Biochemistry](#) and [Microbial Metabolism](#)—assume that the reader already has an understanding of the concepts reviewed here.

Atomic Structure

Life is made up of matter. Matter occupies space and has mass. All matter is composed of **atoms**. All atoms contain **protons**, **electrons**, and **neutrons** ([\[link\]](#)). The only exception is hydrogen (H), which is made of one proton and one electron. A proton is a positively charged particle that resides in the nucleus (the core of the atom) of an atom and has a mass of 1 atomic mass unit (amu) and a charge of +1. An electron is a negatively charged particle that travels in the space around the nucleus. Electrons have a negligible mass and a charge of -1. Neutrons, like protons, reside in the nucleus of an atom. They have a mass of 1 amu and no charge (neutral). The positive (proton) and negative (electron) charges balance each other in a neutral atom, which has a net zero charge. Because protons and neutrons each have a mass of 1 amu, the mass of an atom is equal to the number of protons and neutrons of that atom. The number of electrons does not factor into the overall mass because electron mass is so small.



Atoms are made up of protons and neutrons located within the nucleus and electrons surrounding the nucleus.

Chemical Elements

All matter is composed of atoms of **elements**. Elements have unique physical and chemical properties and are substances that cannot easily be transformed either physically or chemically into other substances. Each element has been given a name, usually derived from Latin or English. The elements also have one- or two-letter symbols representing the name; for example, sodium (Na), gold (Au), and silver (Ag) have abbreviations derived from their original Latin names *natrium*, *aurum*, and *argentum*, respectively. Examples with English abbreviations are carbon (C), hydrogen (H), oxygen (O), and nitrogen (N). A total of 118 different elements (92 of which occur naturally) have been identified and organized into the periodic table of elements. Of the naturally occurring elements, fewer than 30 are found in

organisms on Earth, and four of those (C, H, O, and N) make up approximately 96% of the mass of an organism.[\[footnote\]](#)

Schrijver, Karel, and Iris Schrijver. *Living with the Stars: How the Human Body Is Connected to the Life Cycles of the Earth, the Planets, and the Stars.* Oxford University Press, USA, 2015.

Each unique element is identified by the number of protons in its atomic nucleus. In addition to protons, each element's atomic nucleus contains an equal or greater number of neutrons (with the exception of hydrogen, which has only one proton). The total number of protons per element is described as the **atomic number**, and the combined mass of protons and neutrons is called the **atomic mass** or **mass number**. Therefore, it is possible to determine the number of neutrons by subtracting the atomic number from the mass number.

Isotopes are different forms of the same element that have the same number of protons, but a different number of neutrons. Many elements have several isotopes with one or two commonly occurring isotopes in nature. For example, carbon-12 (^{12}C), the most common isotope of carbon (98.6% of all C found on Earth),[\[footnote\]](#) contains six protons and six neutrons.

Therefore, it has a mass number of 12 (6 protons + 6 neutrons) and an atomic number of 6.

National Oceanic and Atmospheric Administration, “Stable and Radiocarbon Isotopes of Carbon Dioxide.” Web page. Accessed Feb 19, 2016
[<http://www.esrl.noaa.gov/gmd/outreach/isotopes/chemistry.html>]

There are two additional types of isotopes in nature: heavy isotopes, and radioisotopes. Heavy isotopes have one or more extra neutrons while still maintaining a stable atomic nucleus. An example of a heavy isotope is carbon-13 (^{13}C) (1.1% of all carbon).[\[footnote\]](#) ^{13}C has a mass number of 13 (6 protons + 7 neutrons). Since the atomic number of ^{13}C is 6, it is still the element carbon; however, it has more mass than the more common form of the element, ^{12}C , because of the extra neutron in the nucleus. Carbon-14 (^{14}C) (0.0001% of all carbon)[\[footnote\]](#) is an example of a radioisotope. ^{14}C has a mass number of 14 (6 protons + 8 neutrons); however, the extra neutrons in ^{14}C result in an unstable nucleus. This instability leads to the process of radioactive decay. Radioactive decay involves the loss of one or

more neutrons and the release of energy in the form of gamma rays, alpha particles, or beta particles (depending on the isotope).

ibid.

ibid.

Heavy isotopes and radioisotopes of carbon and other elements have proven to be useful in research, industry, and medicine.

Chemical Bonds

There are three types of chemical bonds that are important when describing the interaction of atoms both within and between molecules in microbiology: (1) covalent bonds, which can be either polar or non-polar, (2) ionic bonds, and (3) hydrogen bonds. There are other types of interactions such as *London* dispersion forces and *van der Waals* forces that could also be discussed when describing the physical and chemical properties of the intermolecular interactions of atoms, but we will not include descriptions of these forces here.

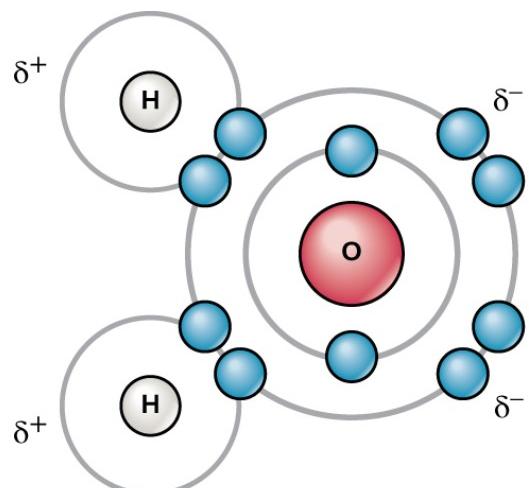
Chemical bonding is determined by the outermost shell of electrons, called the valence electrons (VE), of an atom. The number of VE is important when determining the number and type of chemical bonds an atom will form.

Covalent Bonds

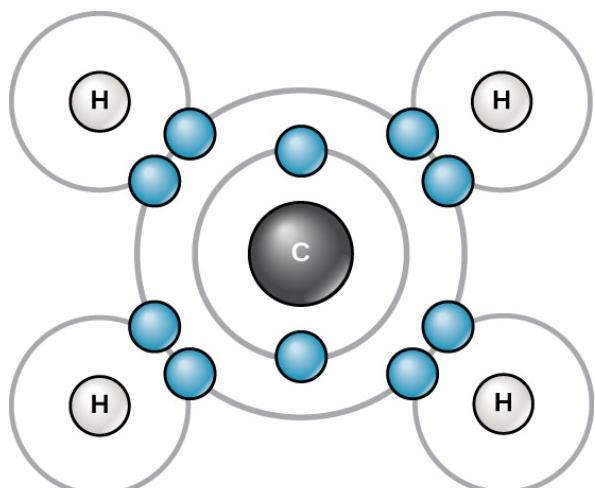
The strongest chemical bond between two or more atoms is a **covalent bond**. These bonds form when an electron is shared between two atoms, and these are the most common form of chemical bond in living organisms. Covalent bonds form between the atoms of elements that make up the biological molecules in our cells. An example of a simple molecule formed with covalent bonds is water, H₂O, with one VE per H atom and 6 VE per O atom. Because of the VE configuration, each H atom is able to accept one additional VE and each O atom is able to accept two additional VE. When sharing electrons, the hydrogen and oxygen atoms that combine to form water molecules become bonded together by covalent bonds ([\[link\]](#)). The electron from the hydrogen atom divides its time between the outer electron

shell of the hydrogen atom and the outermost electron shell of the oxygen atom. To completely fill the outer shell of an oxygen atom, two electrons from two hydrogen atoms are needed, hence the subscript “2” indicating two atoms of H in a molecule of H₂O. This sharing is a lower energy state for all of the atoms involved than if they existed without their outer shells filled.

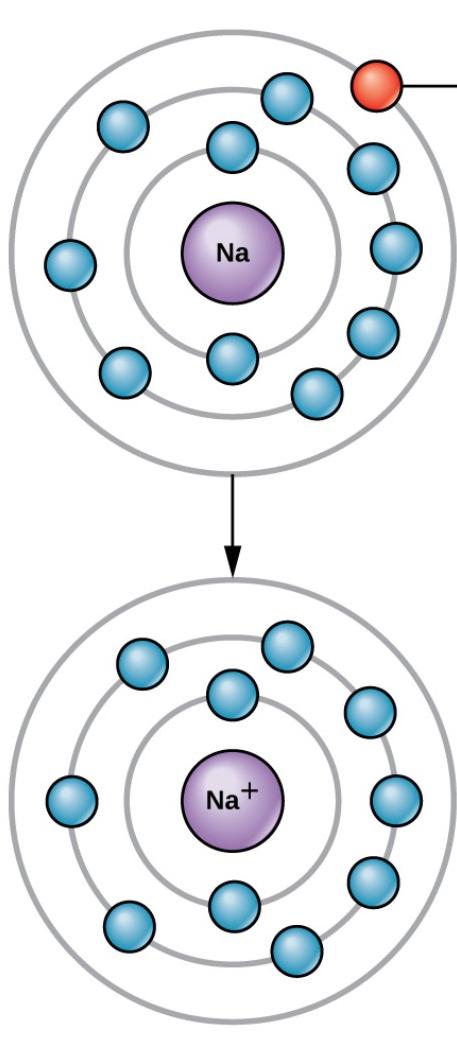
There are two types of covalent bonds: polar and nonpolar. **Nonpolar covalent** bonds form between two atoms of the same or different elements that share the electrons equally ([\[link\]](#)). In a **polar covalent bond**, the electrons shared by the atoms spend more time closer to one nucleus than to the other nucleus. Because of the unequal distribution of electrons between the different nuclei, a slightly positive ($\delta+$) or slightly negative ($\delta-$) charge develops. Water is an example of a molecule formed with **polar covalent bonds** ([\[link\]](#)).



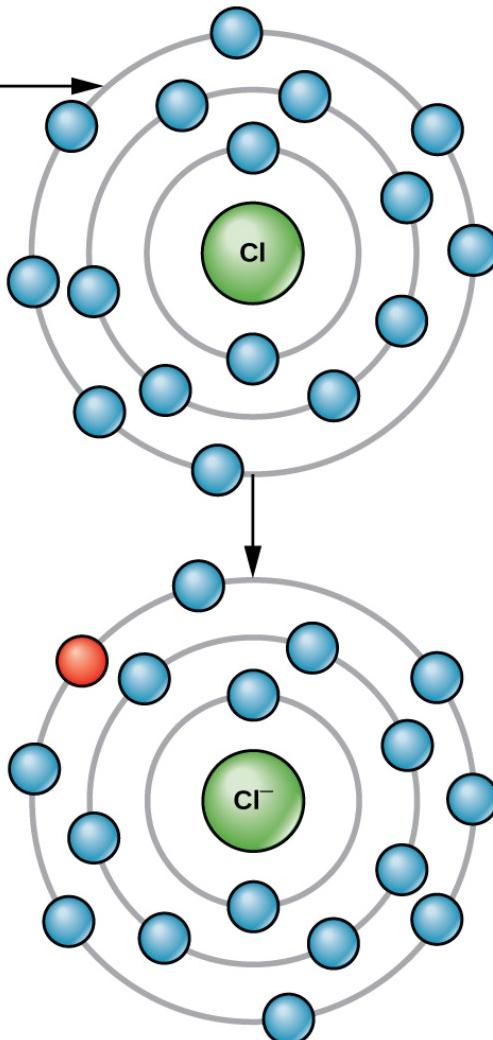
Polar covalent bond



Nonpolar covalent bond



net positive charge



net negative charge

Ionic bond

The water molecule (top left) depicts a polar bond with a slightly positive charge on the hydrogen atoms and a slightly negative charge on the oxygen. Methane (top right) is an example of a nonpolar covalent bond. Sodium chloride (bottom) is a substance formed from ionic bonds between sodium and chlorine.

Ions and Ionic Bonds

When an atom does not contain equal numbers of protons and electrons, it is called an **ion**. Because the number of electrons does not equal the number of protons, each ion has a net charge. Positive ions are formed by losing electrons and are called **cations**. Negative ions are formed by gaining electrons and are called **anions**.

For example, a sodium atom has only has one electron in its outermost shell. It takes less energy for the sodium atom to donate that one electron than it does to accept seven more electrons, which it would need to fill its outer shell. If the sodium atom loses an electron, it now has 11 protons and only 10 electrons, leaving it with an overall charge of +1. It is now called a sodium ion (Na^+).

A chlorine atom has seven electrons in its outer shell. Again, it is more energy efficient for the chlorine atom to gain one electron than to lose seven. Therefore, it will more likely gain an electron to form an ion with 17 protons and 18 electrons, giving it a net negative (-1) charge. It is now called a chloride ion (Cl^-). This movement of electrons from one atom to another is referred to as electron transfer. Because positive and negative charges attract, these ions stay together and form an **ionic bond**, or a bond between ions. When Na^+ and Cl^- ions combine to produce NaCl , an electron from a sodium atom stays with the other seven from the chlorine atom, and the sodium and chloride ions attract each other in a lattice of ions with a net zero charge ([\[link\]](#)).

Polyatomic ions consist of multiple atoms joined by covalent bonds; but unlike a molecule, a polyatomic ion has a positive or negative charge. It behaves as a cation or anion and can therefore form ionic bonds with other ions to form ionic compounds. The atoms in a polyatomic ion may be from the same element or different elements.

[[link](#)] lists some cations and anions that commonly occur in microbiology. Note that this table includes monoatomic as well as polyatomic ions.

Some Common Ions in Microbiology			
Cations		Anions	
sodium	Na^+	chloride	Cl^-
hydrogen	H^+	bicarbonate	HCO_3^-
potassium	K^+	carbonate	CO_3^{2-}
ammonium	NH_4^+	hydrogen sulfate	$\text{H}_2\text{SO}_4^{2-}$
copper (I)	Cu^+	hydrogen sulfide	HS^-
copper (II)	Cu^{2+}	hydroxide	OH^-
iron (II)	Fe^{2+}	hypochlorite	ClO^-
iron (III)	Fe^{3+}	nitrite	NO_2^-
		nitrate	NO_3^-
		peroxide	O_2^{2-}

Some Common Ions in Microbiology

Cations	Anions
	phosphate
	pyrophosphate
	sulfite
	thiosulfate

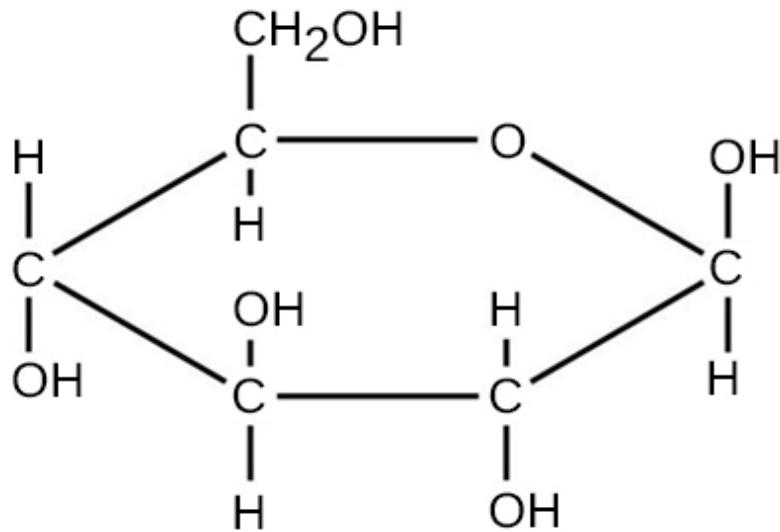
Molecular Formula, Molecular Mass, and the Mole

For molecules formed by covalent bonds, the molecular formula represents the number and types of elemental atoms that compose the molecule. As an example, consider a molecule of glucose, which has the molecular formula C₆H₁₂O₆. This molecular formula indicates that a single molecule of glucose is formed from six carbon atoms, twelve hydrogen atoms, and six oxygen atoms.

The **molecular mass** of a molecule can be calculated using the molecular formula and the atomic mass of each element in the molecule. The number of each type of atom is multiplied by the atomic mass; then the products are added to get the molecular mass. For example the molecular mass of glucose, C₆H₁₂O₆ ([\[link\]](#)), is calculated as:

Equation:

$$\begin{aligned}
 \text{mass of carbon} &= 12 \frac{\text{amu}}{\text{atom}} \times 6 \text{ atoms} = 72 \text{ amu} \\
 \text{mass of hydrogen} &= 1 \frac{\text{amu}}{\text{atom}} \times 12 \text{ atoms} = 12 \text{ amu} \\
 \text{mass of oxygen} &= 16 \frac{\text{amu}}{\text{atom}} \times 6 \text{ atoms} = 96 \text{ amu} \\
 \text{molecular mass of glucose} &= 72 \text{ amu} + 12 \text{ amu} + 96 \text{ amu} = 180 \text{ amu}
 \end{aligned}$$



The molecular structure of glucose showing the numbers of carbon, oxygen, and hydrogen atoms. Glucose has a molecular mass of 180 amu.

The number of entities composing a mole has been experimentally determined to be 6.022×10^{23} , a fundamental constant named **Avogadro's number** (NA) or the Avogadro constant. This constant is properly reported with an explicit unit of “per mole.”

Energy

Thermodynamics refers to the study of energy and energy transfer involving physical matter.

Matter participating in a particular case of energy transfer is called a system, and everything outside of that matter is called the surroundings. There are two types of systems: open and closed. In an **open system**, energy can be exchanged with its surroundings. A **closed system** cannot exchange energy with its surroundings. Biological organisms are open systems. Energy is exchanged between them and their surroundings as they use energy from the sun to perform photosynthesis or consume energy-storing molecules and

release energy to the environment by doing work and releasing heat. Like all things in the physical world, energy is subject to physical laws. In general, energy is defined as the ability to do work, or to create some kind of change. Energy exists in different forms. For example, electrical energy, light energy, and heat energy are all different types of energy. The **first law of thermodynamics**, often referred to as the law of conservation of energy, states that the total amount of energy in the universe is constant and conserved. Energy exists in many different forms. According to the first law of thermodynamics, energy may be transferred from place to place or transformed into different forms, but it cannot be created or destroyed.

The challenge for all living organisms is to obtain energy from their surroundings in forms that they can transfer or transform into usable energy to do work. Microorganisms have evolved to meet this challenge. Chemical energy stored within organic molecules such as sugars and fats is transferred and transformed through a series of cellular chemical reactions into energy within molecules of ATP. Energy in ATP molecules is easily accessible to do work. Examples of the types of work that cells need to do include building complex molecules, transporting materials, powering the motion of cilia or flagella, and contracting muscle fibers to create movement.

A microorganism's primary tasks of obtaining, transforming, and using energy to do work may seem simple. However, the **second law of thermodynamics** explains why these tasks are more difficult than they appear. All energy transfers and transformations are never completely efficient. In every energy transfer, some amount of energy is lost in a form that is unusable. In most cases, this form is **heat energy**. Thermodynamically, heat energy is defined as the energy transferred from one system to another that is not work. For example, some energy is lost as heat energy during cellular metabolic reactions.

The more energy that is lost by a system to its surroundings, the less ordered and more random the system is. Scientists refer to the measure of randomness or disorder within a system as **entropy**. High entropy means high disorder and low energy. Molecules and chemical reactions have varying entropy as well. For example, entropy increases as molecules at a high concentration in one place diffuse and spread out. The second law of thermodynamics says that energy will always be lost as heat in energy

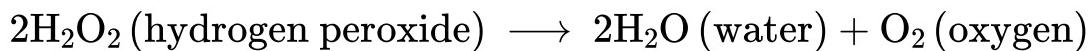
transfers or transformations. Microorganisms are highly ordered, requiring constant energy input to be maintained in a state of low entropy.

Chemical Reactions

Chemical reactions occur when two or more atoms bond together to form molecules or when bonded atoms are broken apart. The substances used in a chemical reaction are called the **reactants** (usually found on the left side of a chemical equation), and the substances produced by the reaction are known as the **products** (usually found on the right side of a chemical equation). An arrow is typically drawn between the reactants and products to indicate the direction of the chemical reaction; this direction is not always a “one-way street.”

An example of a simple chemical reaction is the breaking down of hydrogen peroxide molecules, each of which consists of two hydrogen atoms bonded to two oxygen atoms (H_2O_2). The reactant hydrogen peroxide is broken down into water, containing one oxygen atom bound to two hydrogen atoms (H_2O), and oxygen, which consists of two bonded oxygen atoms (O_2). In the equation below, the reaction includes two hydrogen peroxide molecules and two water molecules. This is an example of a balanced chemical equation, wherein the number of atoms of each element is the same on each side of the equation. According to the law of conservation of matter, the number of atoms before and after a chemical reaction should be equal, such that no atoms are, under normal circumstances, created or destroyed.

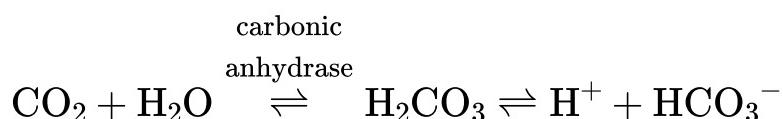
Equation:



Some chemical reactions, such as the one shown above, can proceed in one direction until the reactants are all used up. Equations that describe these reactions contain a unidirectional arrow and are irreversible. **Reversible reactions** are those that can go in either direction. In reversible reactions, reactants are turned into products, but when the concentration of product rises above a certain threshold (characteristic of the particular reaction), some of these products will be converted back into reactants; at this point, the designations of products and reactants are reversed. The changes in

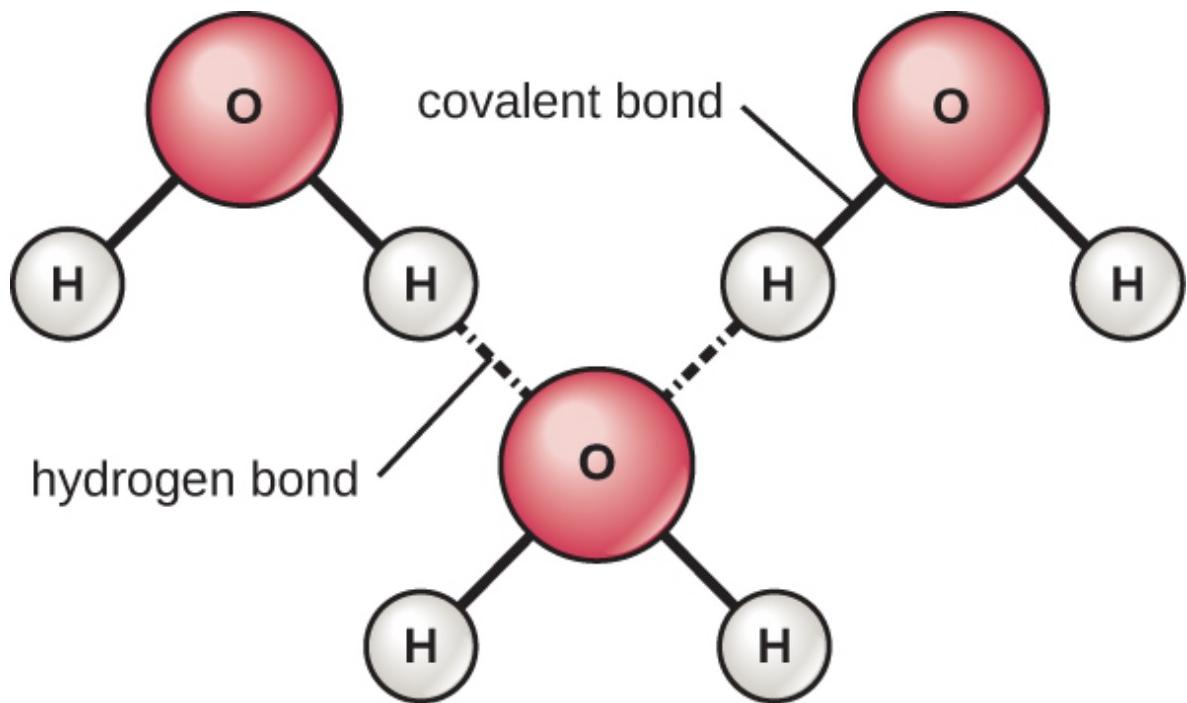
concentration continue until a certain relative balance in concentration between reactants and products occurs—a state called **chemical equilibrium**. At this point, both the forward and reverse reactions continue to occur, but they do so at the same rate, so the concentrations of reactants and products do not change. These situations of reversible reactions are often denoted by a chemical equation with a double-headed arrow pointing towards both the reactants and products. For example, when carbon dioxide dissolves in water, it can do so as a gas dissolved in water or by reacting with water to produce carbonic acid. In the cells of some microorganisms, the rate of carbonic acid production is accelerated by the enzyme carbonic anhydrase, as indicated in the following equation:

Equation:



Properties of Water and Solutions

The hydrogen and oxygen atoms within water molecules form polar covalent bonds. There is no overall charge to a water molecule, but there is one δ^+ on each hydrogen atom and two δ^- on the oxygen atom. Each water molecule attracts other water molecules because of the positive and negative charges in the different parts of the molecule ([\[link\]](#)). Water also attracts other polar molecules (such as sugars), forming hydrogen bonds. When a substance readily forms hydrogen bonds with water, it can dissolve in water and is referred to as **hydrophilic** (“water-loving”). Hydrogen bonds are not readily formed with nonpolar substances like oils and fats. These nonpolar compounds are **hydrophobic** (“water-fearing”) and will orient away from and avoid water.



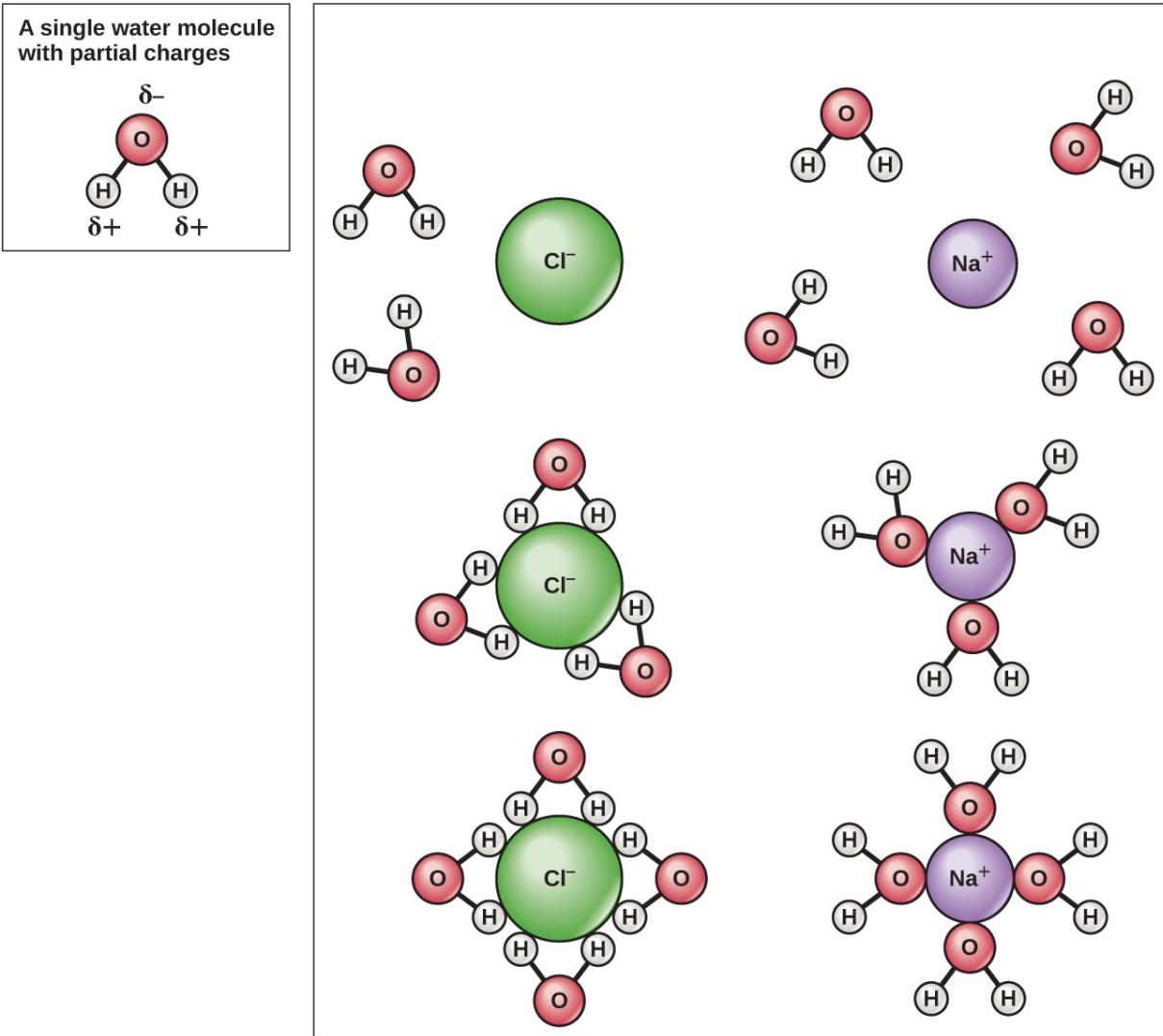
Hydrogen bonds form between slightly positive ($\delta+$) and slightly negative ($\delta-$) charges of polar covalent molecules such as water.

The hydrogen bonds in water allow it to absorb and release heat energy more slowly than many other substances. This means that water moderates temperature changes within organisms and in their environments. As energy input continues, the balance between hydrogen-bond formation and breaking swings toward fewer hydrogen bonds: more bonds are broken than are formed. This process results in the release of individual water molecules at the surface of the liquid (such as a body of water, the leaves of a plant, or the skin of an organism) in a process called **evaporation**.

Conversely, as molecular motion decreases and temperatures drop, less energy is present to break the hydrogen bonds between water molecules. These bonds remain intact and begin to form a rigid, lattice-like structure (e.g., ice). When frozen, ice is less dense (the molecules are farther apart) than liquid water. This means that ice floats on the surface of a body of water. In lakes, ponds, and oceans, ice will form on the surface of the water, creating an insulating barrier to protect the animal and plant life beneath

from freezing in the water. If this did not happen, plants and animals living in water would freeze in a block of ice and could not move freely, making life in cold temperatures difficult or impossible.

Because water is polar, with slight positive and negative charges, ionic compounds and polar molecules can readily dissolve in it. Water is, therefore, what is referred to as a solvent—a substance capable of dissolving another substance. The charged particles will form hydrogen bonds with a surrounding layer of water molecules. This is referred to as a **sphere of hydration** and serves to keep the ions separated or dispersed in the water ([\[link\]](#)). These spheres of hydration are also referred to as hydration shells. The polarity of the water molecule makes it an effective solvent and is important in its many roles in living systems.



When table salt (NaCl) is mixed in water, spheres of hydration form around the ions.

The ability of insects to float on and skate across pond water results from the property of **cohesion**. In cohesion, water molecules are attracted to each other (because of hydrogen bonding), keeping the molecules together at the liquid-air (gas) interface. Cohesion gives rise to surface tension, the capacity of a substance to withstand rupture when placed under tension or stress.

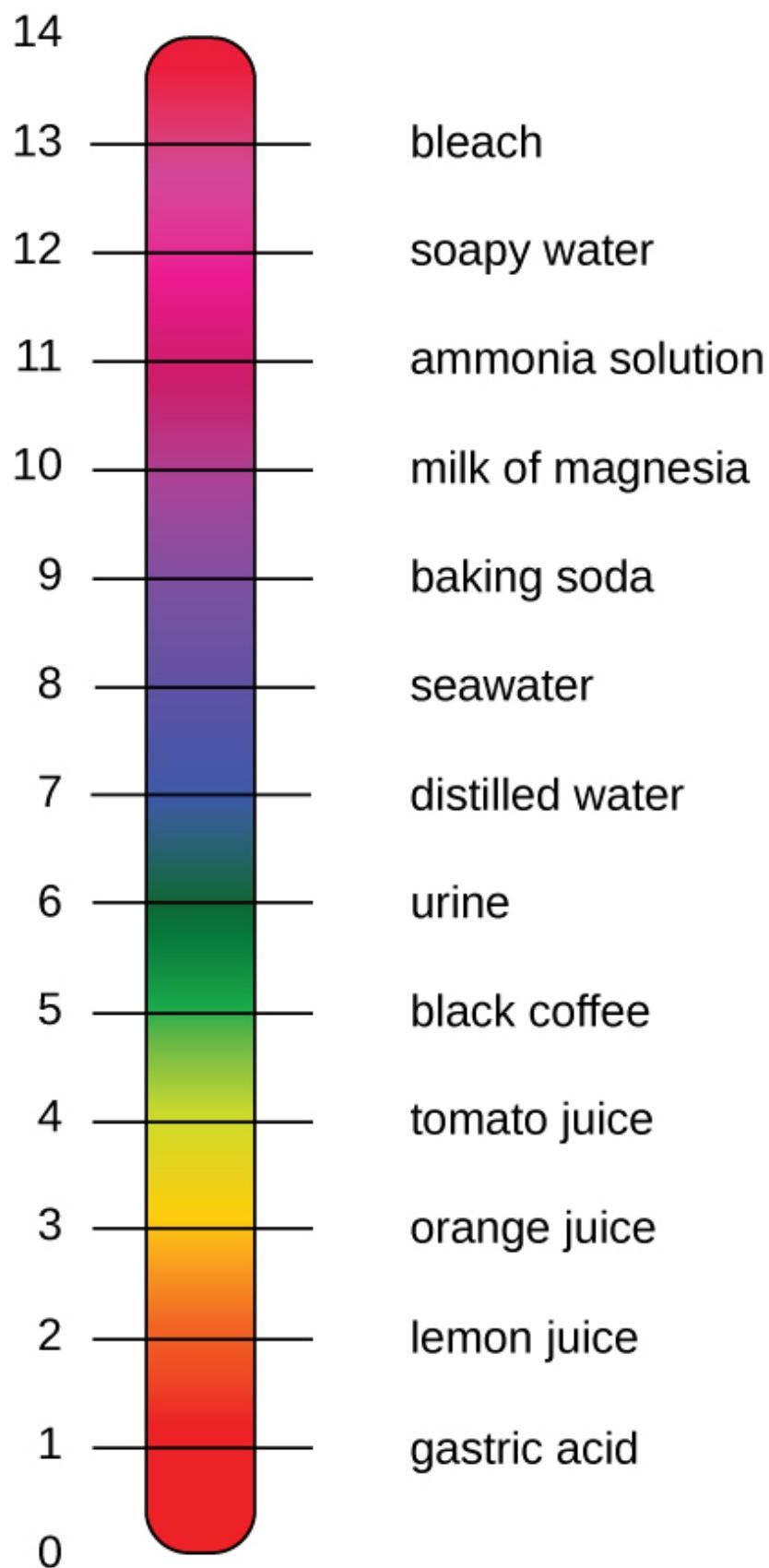
These cohesive forces are also related to water's property of **adhesion**, or the attraction between water molecules and other molecules. This is observed

when water “climbs” up a straw placed in a glass of water. You will notice that the water appears to be higher on the sides of the straw than in the middle. This is because the water molecules are attracted to the straw and therefore adhere to it.

Cohesion and adhesion are also factors in bacterial colonies and biofilm formation. Cohesion keeps the colony intact (helps it “stick” to a surface), while adhesion keeps the cells adhered to each other. Cohesive and adhesive forces are important for sustaining life. For example, because of these forces, water in natural surroundings provides the conditions necessary to allow bacterial and archaeal cells to adhere and accumulate on surfaces.

Acids and Bases

The **pH** of a solution is a measure of hydrogen ion (H^+) and hydroxide ion (OH^-) concentrations and is described as **acidity** or **alkalinity**, respectively. Acidity and alkalinity (also referred to as basicity) can be measured and calculated. pH can be simply represented by the mathematic equation, $pH = -\log_{10}[H^+]$. On the left side of the equation, the "p" means "the negative logarithm of" and the H represents the $[H^+]$. On the right side of the equation, $[H^+]$ is the concentration of H^+ in moles/L. What is not represented in this simple equation is the contribution of the OH^- , which also participates in acidity or alkalinity. Calculation of pH results in a number range of 0 to 14 called the pH scale ([\[link\]](#)). A pH value between 0 and 6.9 indicates an acid. It is also referred to as a low pH, due to a high $[H^+]$ and low $[OH^-]$ concentration. A pH value between 7.1 and 14 indicates an alkali or base. It is also referred to as a high pH, due to a low $[H^+]$ and high $[OH^-]$ concentration. A pH of 7 is described as a neutral pH and occurs when $[H^+]$ equals $[OH^-]$.



The pH scale measures the concentration of hydrogen ions $[H^+]$ and $[OH^-]$ in a substance.

(credit: modification of work by Edward Stevens)

A change of one unit on the pH scale represents a change in the $[H^+]$ by a factor of 10, a change in two units represents a change in the $[H^+]$ by a factor of 100. Thus, small changes in pH represent large changes in $[H^+]$.

Mathematical Basics

Squares and Other Powers

An exponent, or a power, is mathematical shorthand for repeated multiplications. For example, the exponent “2” means to multiply the base for that exponent by itself (in the example here, the base is “5”):

Equation:

$$5^2 = 5 \times 5 = 25$$

The exponent is “2” and the base is the number “5.” This expression (multiplying a number by itself) is also called a square. Any number raised to the power of 2 is being squared. Any number raised to the power of 3 is being cubed:

Equation:

$$5^3 = 5 \times 5 \times 5 = 125$$

A number raised to the fourth power is equal to that number multiplied by itself four times, and so on for higher powers. In general:

Equation:

$$n^x = n \times n^{x-1}$$

Calculating Percents

A percent is a way of expressing a fractional amount of something using a whole divided into 100 parts. A percent is a ratio whose denominator is 100. We use the percent symbol, %, to show percent. Thus, 25% means a ratio of $\frac{25}{100}$, 3% means a ratio of $\frac{3}{100}$, and 100 % percent means $\frac{100}{100}$, or a whole.

Converting Percents

A percent can be converted to a fraction by writing the value of the percent as a fraction with a denominator of 100 and simplifying the fraction if possible.

Equation:

$$25\% = \frac{25}{100} = \frac{1}{4}$$

A percent can be converted to a decimal by writing the value of the percent as a fraction with a denominator of 100 and dividing the numerator by the denominator.

Equation:

$$10\% = \frac{10}{100} = 0.10$$

To convert a decimal to a percent, write the decimal as a fraction. If the denominator of the fraction is not 100, convert it to a fraction with a denominator of 100, and then write the fraction as a percent.

Equation:

$$0.833 = \frac{833}{1000} = \frac{83.3}{100} = 83.3\%$$

To convert a fraction to a percent, first convert the fraction to a decimal, and then convert the decimal to a percent.

Equation:

$$\frac{3}{4} = 0.75 = \frac{75}{100} = 75\%$$

Suppose a researcher finds that 15 out of 23 students in a class are carriers of *Neisseria meningitidis*. What percentage of students are carriers? To find

this value, first express the numbers as a fraction.

Equation:

$$\frac{\text{carriers}}{\text{total students}} = \frac{15}{23}$$

Then divide the numerator by the denominator.

Equation:

$$\frac{15}{23} = 15 \div 23 \approx 0.65$$

Finally, to convert a decimal to a percent, multiply by 100.

Equation:

$$0.65 \times 100 = 65\%$$

The percent of students who are carriers is 65%.

You might also get data on occurrence and non-occurrence; for example, in a sample of students, 9 tested positive for *Toxoplasma* antibodies, while 28 tested negative. What is the percentage of seropositive students? The first step is to determine the “whole,” of which the positive students are a part. To do this, sum the positive and negative tests.

Equation:

$$\text{positive} + \text{negative} = 9 + 28 = 37$$

The whole sample consisted of 37 students. The fraction of positives is:

Equation:

$$\frac{\text{positive}}{\text{total students}} = \frac{9}{37}$$

To find the percent of students who are carriers, divide the numerator by the denominator and multiply by 100.

Equation:

$$\frac{9}{37} = 9 \div 37 \approx 0.24$$
$$0.24 \times 100 = 24\%$$

The percent of positive students is about 24%.

Another way to think about calculating a percent is to set up equivalent fractions, one of which is a fraction with 100 as the denominator, and cross-multiply. The previous example would be expressed as:

Equation:

$$\frac{9}{37} = \frac{x}{100}$$

Now, cross multiply and solve for the unknown:

Equation:

$$9 \times 100 = 37x$$
$$\frac{9 \times 100}{37} = x \quad \text{Divide both sides by 37}$$
$$\frac{900}{37} = x \quad \text{Multiply}$$
$$24 \approx x \quad \text{Divide}$$

The answer, rounded, is the same.

Multiplying and Dividing by Tens

In many fields, especially in the sciences, it is common to multiply decimals by powers of 10. Let's see what happens when we multiply 1.9436 by some powers of 10.

Equation:

$$\begin{aligned}
 1.9436(10) &= 19.436 \\
 1.9436(100) &= 194.36 \\
 1.9436(1000) &= 1943.6
 \end{aligned}$$

The number of places that the decimal point moves is the same as the number of zeros in the power of ten. [\[link\]](#) summarizes the results.

Multiply by	Zeros	Decimal point moves . . .
10	1	1 place to the right
100	2	2 places to the right
1,000	3	3 places to the right
10,000	4	4 places to the right

We can use this pattern as a shortcut to multiply by powers of ten instead of multiplying using the vertical format. We can count the zeros in the power of 10 and then move the decimal point that same number of places to the right.

So, for example, to multiply 45.86 by 100, move the decimal point 2 places to the right.

$$45.86 \times 100 = 4586.$$


Sometimes when we need to move the decimal point, there are not enough decimal places. In that case, we use zeros as placeholders. For example,

let's multiply 2.4 by 100. We need to move the decimal point 2 places to the right. Since there is only one digit to the right of the decimal point, we must write a 0 in the hundredths place.

$$2.4 \times 100 = 240.$$


When dividing by powers of 10, simply take the opposite approach and move the decimal to the left by the number of zeros in the power of ten.

Let's see what happens when we divide 1.9436 by some powers of 10.

Equation:

$$\begin{aligned}1.9436 \div 10 &= 0.19436 \\1.9436 \div 100 &= 0.019436 \\1.9436 \div 1000 &= 0.0019436\end{aligned}$$

If there are insufficient digits to move the decimal, add zeroes to create places.

Scientific Notation

Scientific notation is used to express very large and very small numbers as a product of two numbers. The first number of the product, the digit term, is usually a number not less than 1 and not greater than 10. The second number of the product, the exponential term, is written as 10 with an exponent. Some examples of scientific notation are given in [\[link\]](#).

Standard Notation

Scientific Notation

Standard Notation	Scientific Notation
1000	1×10^3
100	1×10^2
10	1×10^1
1	1×10^0
0.1	1×10^{-1}
0.01	1×10^{-2}

Scientific notation is particularly useful notation for very large and very small numbers, such as $1,230,000,000 = 1.23 \times 10^9$, and $0.00000000036 = 3.6 \times 10^{-10}$.

Expressing Numbers in Scientific Notation

Converting any number to scientific notation is straightforward. Count the number of places needed to move the decimal next to the left-most non-zero digit: that is, to make the number between 1 and 10. Then multiply that number by 10 raised to the number of places you moved the decimal. The exponent is positive if you moved the decimal to the left and negative if you moved the decimal to the right. So

Equation:

$$2386 = 2.386 \times 1000 = 2.386 \times 10^3$$

and

Equation:

$$0.123 = 1.23 \times 0.1 = 1.23 \times 10^{-1}$$

The power (exponent) of 10 is equal to the number of places the decimal is shifted.

Logarithms

The common logarithm (log) of a number is the power to which 10 must be raised to equal that number. For example, the common logarithm of 100 is 2, because 10 must be raised to the second power to equal 100. Additional examples are in [\[link\]](#).

Number	Exponential Form	Common Logarithm
1000	10^3	3
10	10^1	1
1	10^0	0
0.1	10^{-1}	-1
0.001	10^{-3}	-3

To find the common logarithm of most numbers, you will need to use the LOG button on a calculator.

Rounding and Significant Digits

In reporting numerical data obtained via measurements, we use only as many significant figures as the accuracy of the measurement warrants. For example, suppose a microbiologist using an automated cell counter determines that there are 525,341 bacterial cells in a one-liter sample of

river water. However, she records the concentration as 525,000 cells per liter and uses this rounded number to estimate the number of cells that would likely be found in 10 liters of river water. In this instance, the last three digits of the measured quantity are not considered *significant*. They are rounded to account for variations in the number of cells that would likely occur if more samples were measured.

The importance of significant figures lies in their application to fundamental computation. In addition and subtraction, the sum or difference should contain as many digits to the right of the decimal as that in the *least* certain (indicated by underscoring in the following example) of the numbers used in the computation.

Suppose a microbiologist wishes to calculate the total mass of two samples of agar.

Equation:

$$\begin{array}{r} 4.\underline{3}83 \text{ g} \\ 3.00\underline{2}1 \text{ g} \\ \hline 7.38\underline{5} \text{ g} \end{array}$$

The least certain of the two masses has three decimal places, so the sum must have three decimal places.

In multiplication and division, the product or quotient should contain no more digits than in the factor containing the *least* number of significant figures. Suppose the microbiologist would like to calculate how much of a reagent would be present in 6.6 mL if the concentration is 0.638 g/mL.

Equation:

$$0.63\underline{8} \frac{\text{g}}{\text{mL}} \times 6.\underline{6} \text{ mL} = 4.1 \text{ g}$$

Again, the answer has only one decimal place because this is the accuracy of the least accurate number in the calculation.

When rounding numbers, increase the retained digit by 1 if it is followed by a number larger than 5 (“round up”). Do not change the retained digit if the digits that follow are less than 5 (“round down”). If the retained digit is followed by 5, round up if the retained digit is odd, or round down if it is even (after rounding, the retained digit will thus always be even).

Generation Time

It is possible to write an equation to calculate the cell numbers at any time if the number of starting cells and doubling time are known, as long as the cells are dividing at a constant rate. We define N_0 as the starting number of bacteria, the number at time $t = 0$. N_i is the number of bacteria at time $t = i$, an arbitrary time in the future. Finally we will set j equal to the number of generations, or the number of times the cell population doubles during the time interval. Then we have,

Equation:

$$N_i = N_0 \times 2^j$$

This equation is an expression of growth by binary fission.

In our example, $N_0 = 4$, the number of generations, j , is equal to 3 after 90 minutes because the generation time is 30 minutes. The number of cells can be estimated from the following equation:

Equation:

$$\begin{aligned} N_i &= N_0 \times 2^j \\ N_{90} &= 4 \times 2^3 \\ N_{90} &= 4 \times 8 = 32 \end{aligned}$$

The number of cells after 90 minutes is 32.

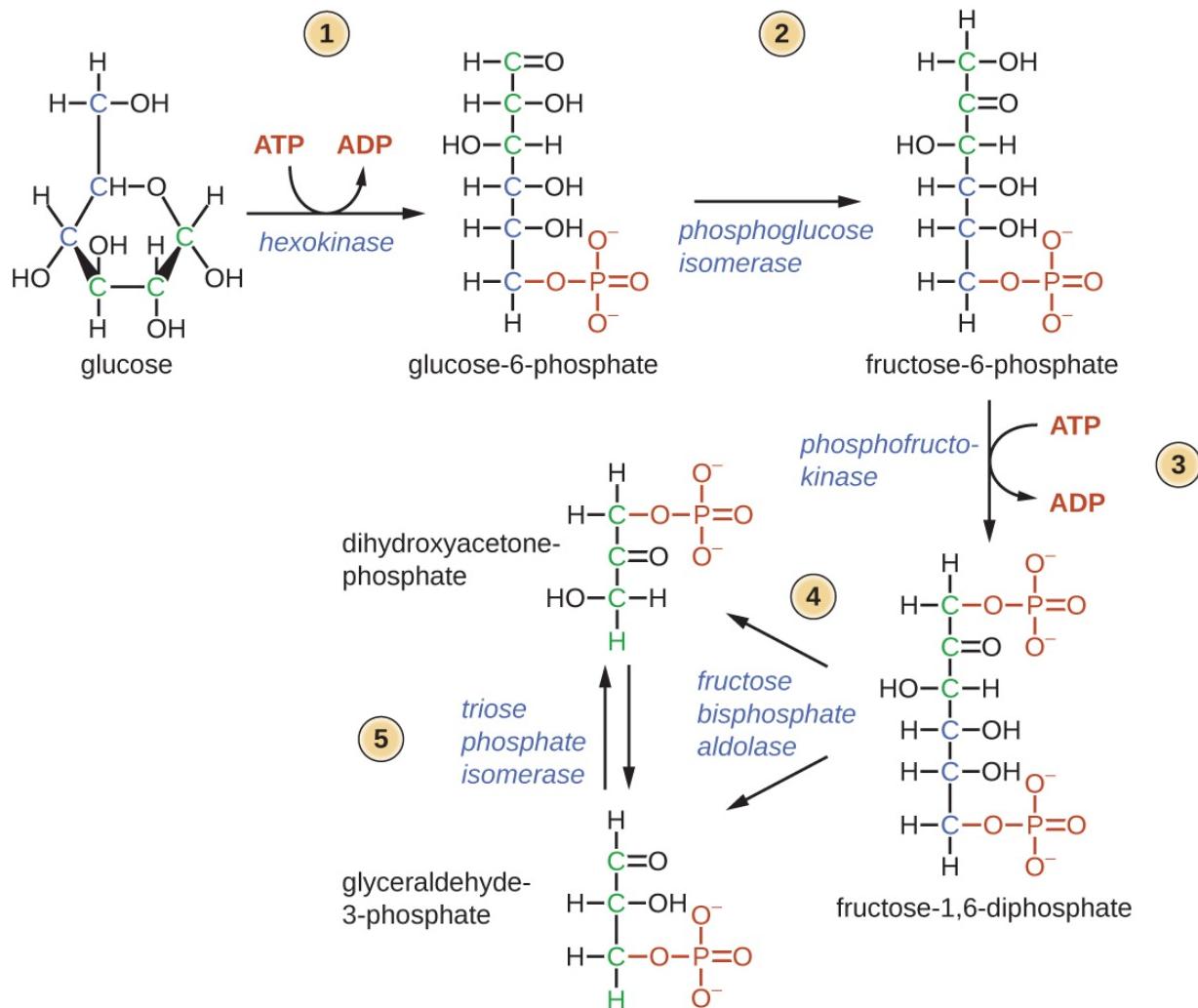
Most Probable Number

The table in [\[link\]](#) contains values used to calculate the most probable number example given in [How Microbes Grow](#).

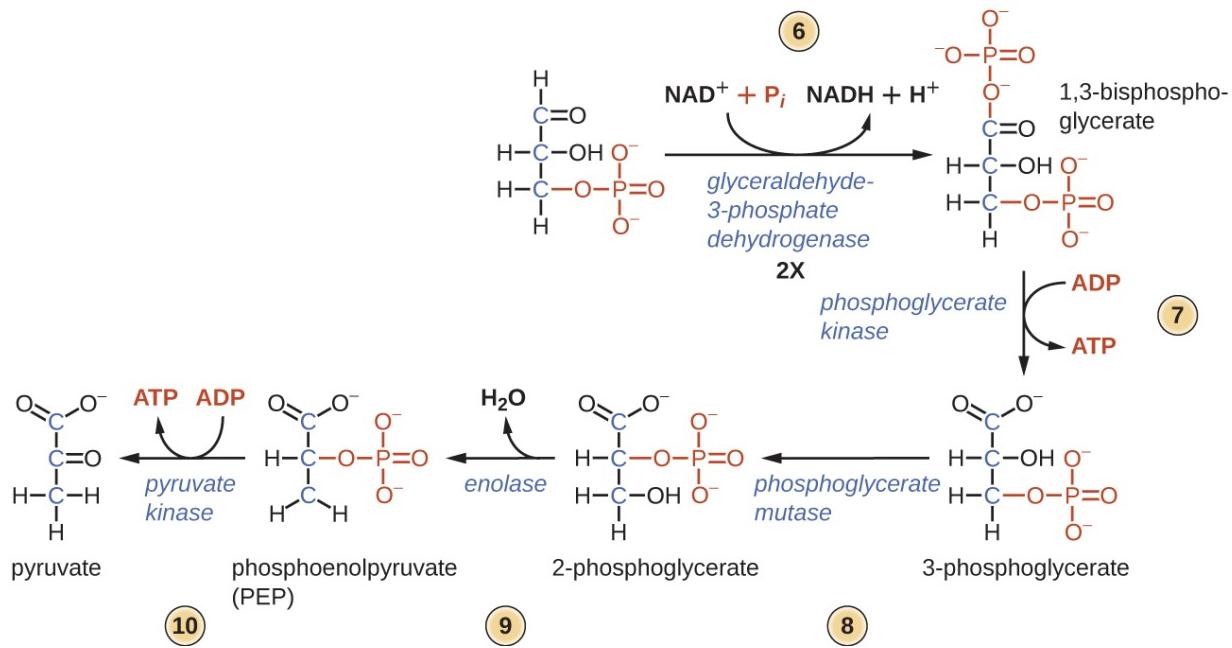
Most Probable Number Table					
Number of tubes giving a positive reaction for a 5-tube set			MPN (per 100 ml)	95% Confidence Limits	
10 ml	1 ml	0.1 ml		Low	High
0	0	0	<2	<1	7
0	1	0	2	<1	7
0	2	0	4	<1	11
1	0	0	2	<1	7
1	0	1	4	<1	11
1	1	0	4	<1	11
1	1	1	6	<1	15
2	0	0	5	<1	13
2	0	1	7	1	17
2	1	0	7	1	17
2	1	1	9	2	21
2	2	0	9	2	21
2	3	0	12	3	28
3	0	0	8	1	19
3	0	1	11	2	25
3	1	0	11	2	25
3	1	1	14	4	34
3	2	0	14	4	34
3	2	1	17	5	46
3	3	0	17	5	46
4	0	0	13	3	31
4	0	1	17	5	46
4	1	0	17	5	46
4	1	1	21	7	63
4	1	2	26	9	78
4	2	0	22	7	67
4	2	1	26	9	78
4	3	0	27	9	80
4	3	1	33	11	93
4	4	0	34	12	93
5	0	0	23	7	70
5	0	1	31	11	89
5	0	2	43	15	110
5	1	0	33	11	93
5	1	1	46	16	120
5	1	2	63	21	150
5	2	0	49	17	130
5	2	1	70	23	170
5	2	2	94	28	220
5	3	0	79	25	190
5	3	1	110	31	250
5	3	2	140	37	340
5	3	3	180	44	500

Metabolic Pathways

Glycolysis

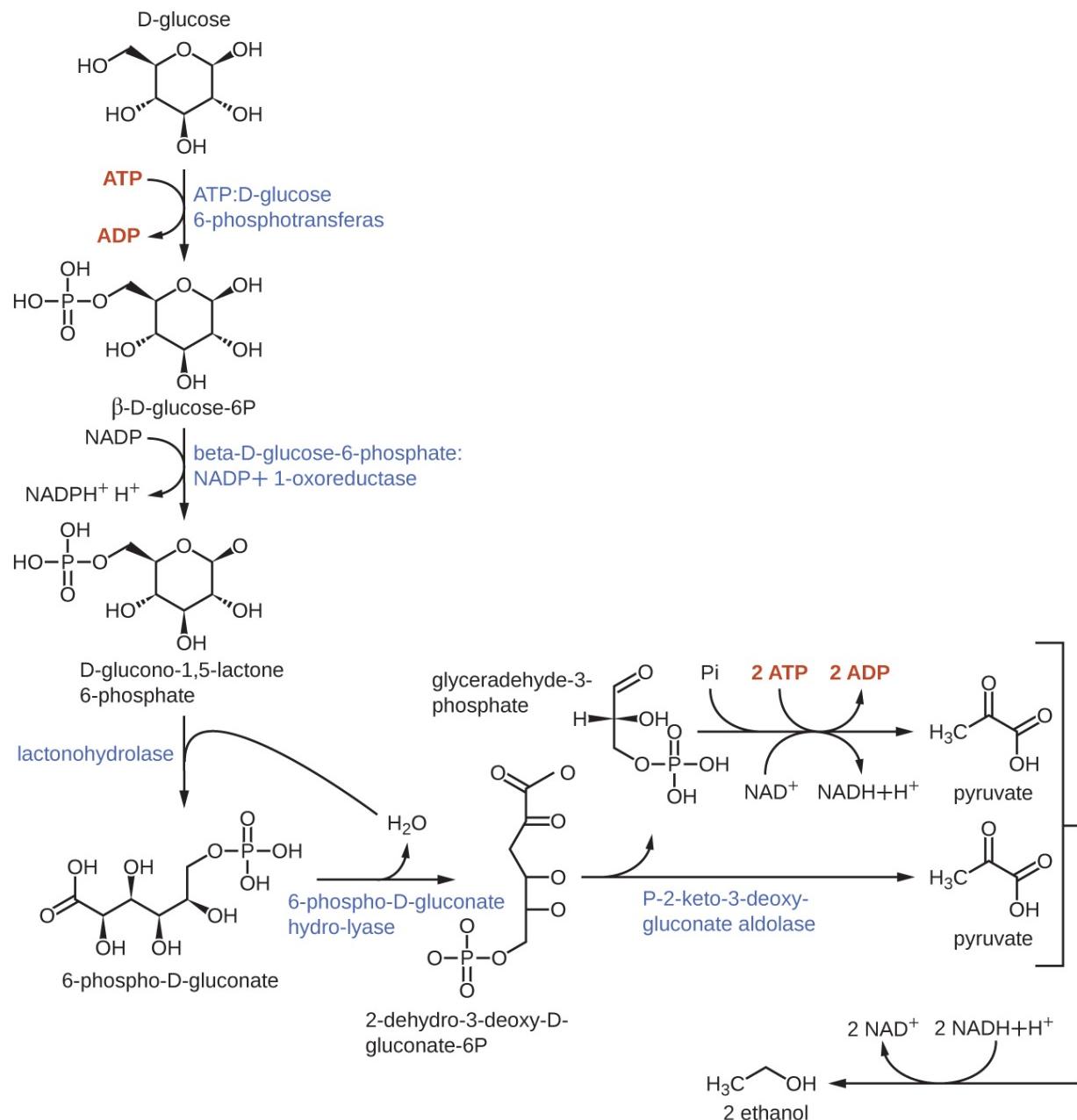


The first half of glycolysis uses two ATP molecules in the phosphorylation of glucose, which is then split into two three-carbon molecules.



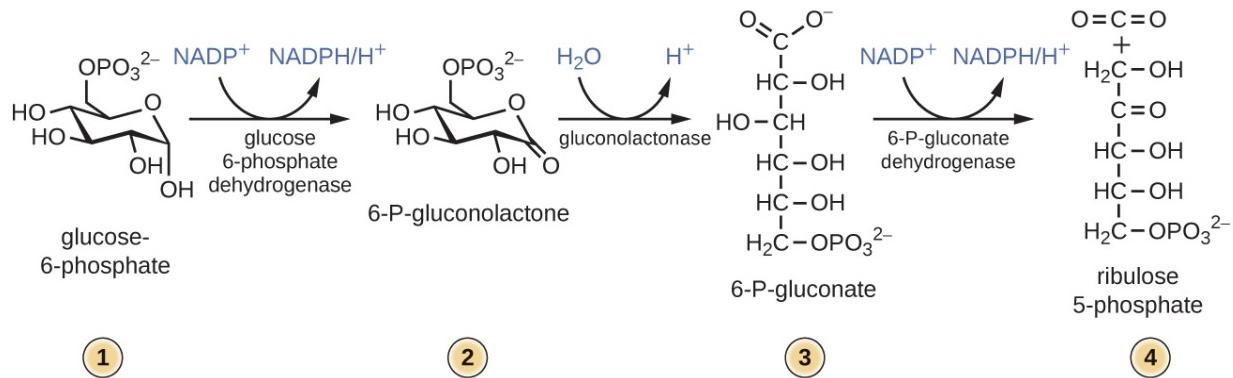
The second half of glycolysis involves phosphorylation without ATP investment (step 6) and produces two NADH and four ATP molecules per glucose.

Entner–Doudoroff Pathway



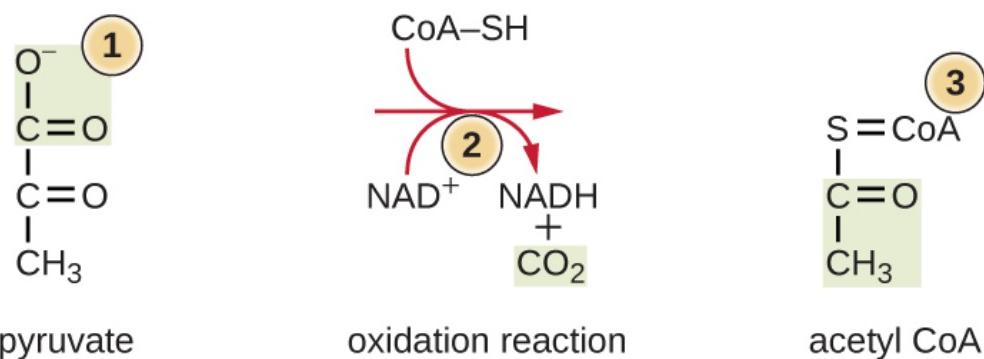
The Entner–Doudoroff Pathway is a metabolic pathway that converts glucose to ethanol and nets one ATP.

The Pentose-Phosphate Pathway



The pentose phosphate pathway, also called the phosphogluconate pathway and the hexose monophosphate shunt, is a metabolic pathway parallel to glycolysis that generates NADPH and five-carbon sugars as well as ribose 5-phosphate, a precursor for the synthesis of nucleotides from glucose.

TCA Cycle

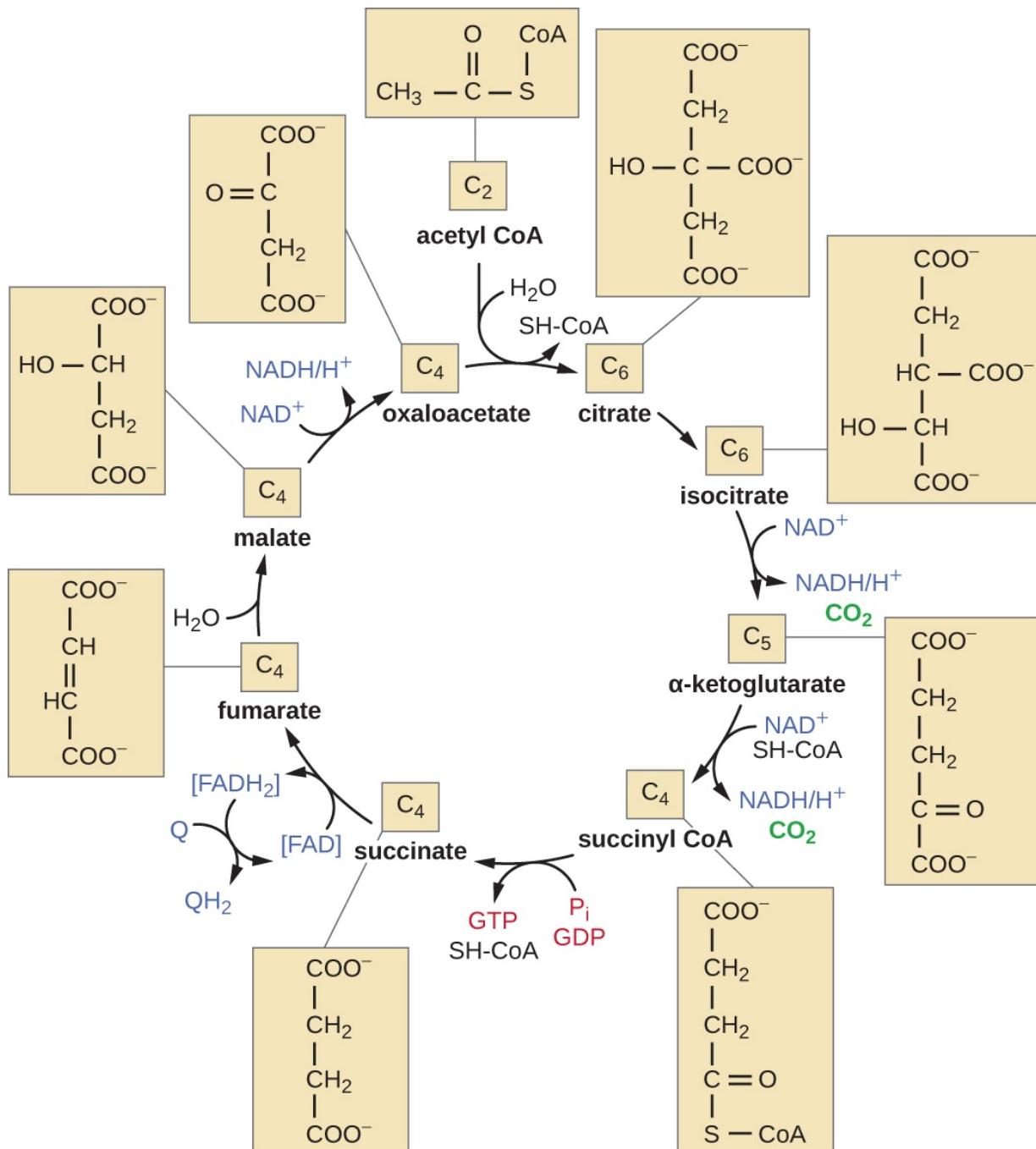


1 A carboxyl group is removed from pyruvate, releasing carbon dioxide.

2 NAD⁺ is reduced to NADH.

3 An acetyl group is transferred to coenzyme A, resulting in acetyl CoA.

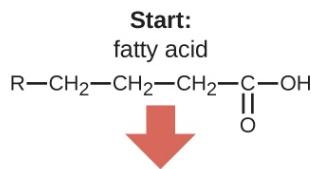
into one acetyl (2C) group plus one carbon dioxide (CO_2). The acetyl group is attached to a Coenzyme A carrier that transports the acetyl group to the site of the Krebs cycle. In the process, one molecule of NADH is formed.



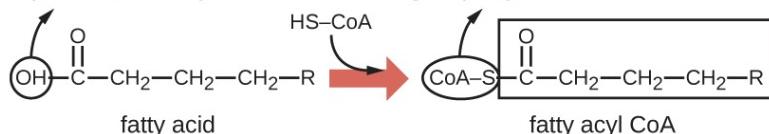
In the citric acid cycle, the acetyl group from acetyl CoA is attached to a four-carbon oxaloacetate molecule to form a six-carbon citrate molecule. Through a series of steps, citrate is oxidized, releasing two carbon dioxide molecules for each acetyl group fed into the cycle. In the process, three NADH, one FADH_2 , and one ATP or GTP (depending on the cell type) is produced by substrate-level phosphorylation. Because the final product of the citric acid cycle is

also the first reactant, the cycle runs continuously in the presence of sufficient reactants. (credit: modification of work by “Yikrazuul”/Wikimedia Commons)

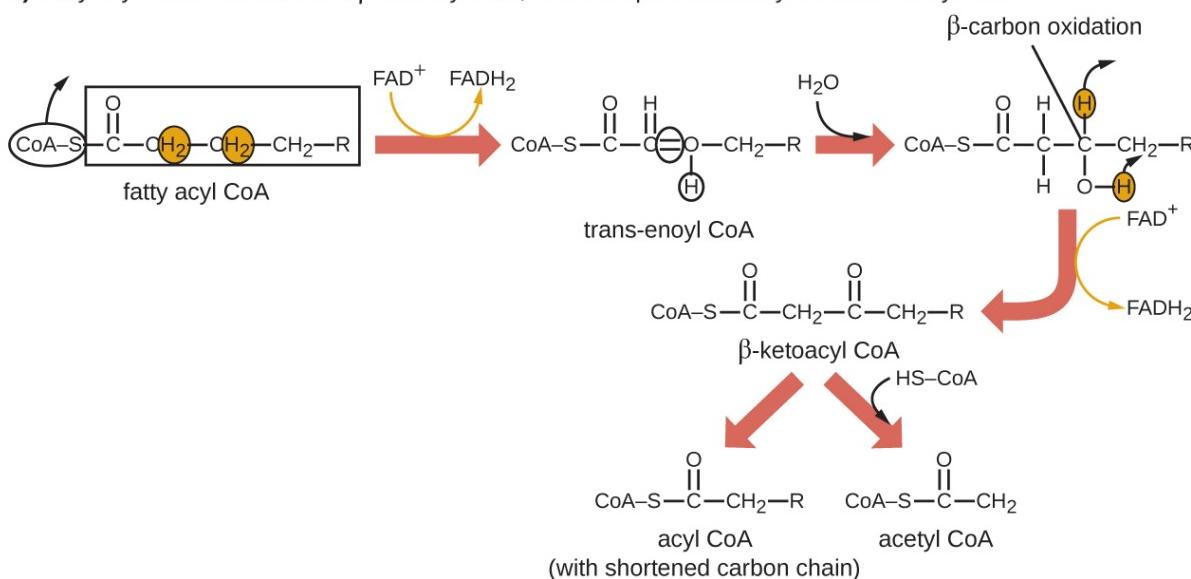
Beta Oxidation



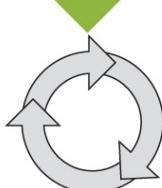
1) Fatty acid couples with Coenzyme A carrier forming fatty acyl CoA



2) Fatty acyl CoA is converted to β -ketoacyl CoA, which is split into an acyl CoA and acetyl CoA

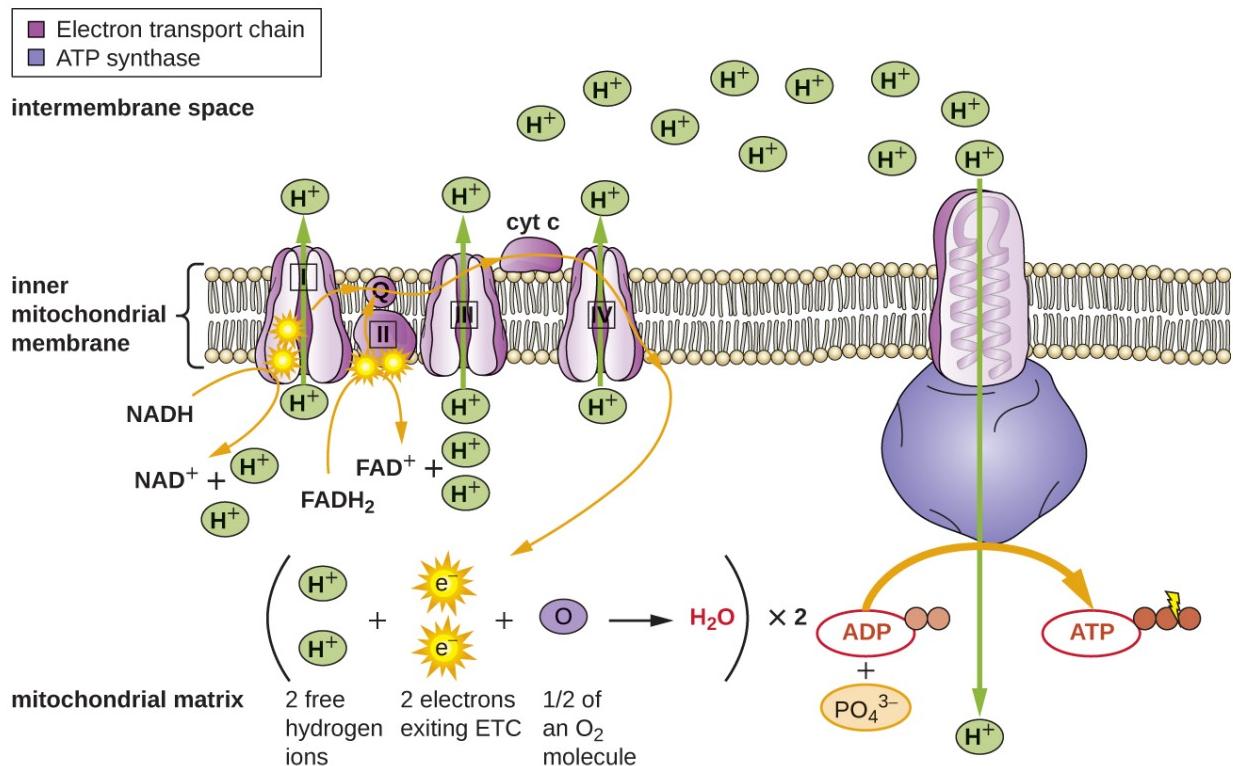


End:
acetyl enters
Krebs cycle



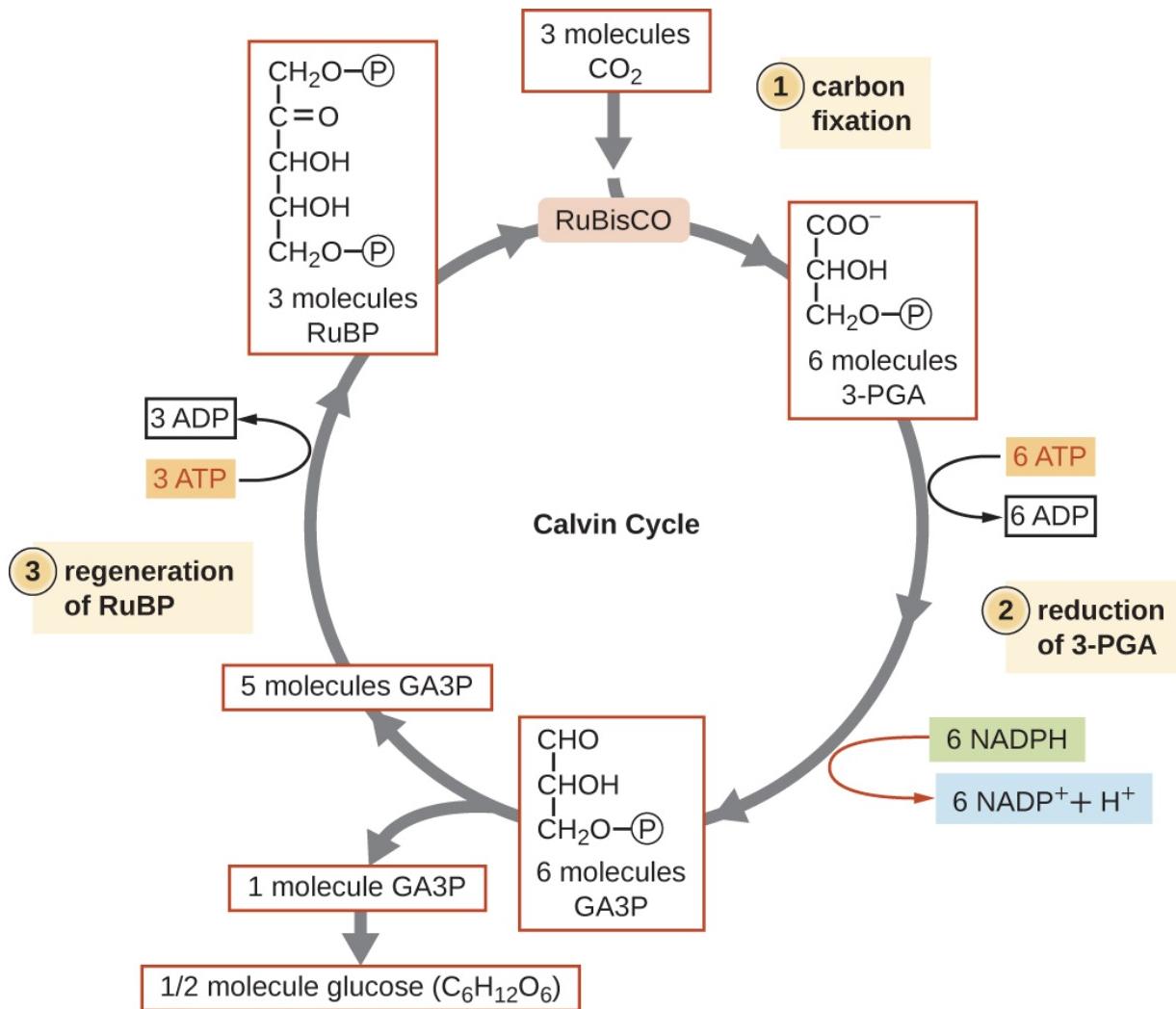
During fatty acid oxidation, triglycerides can be broken down into 2C acetyl groups that can enter the Krebs cycle and be used as a source of energy when glucose levels are low.

Electron Transport Chain and Oxidative Phosphorylation



The electron transport chain is a series of electron carriers and ion pumps that are used to pump H⁺ ions across a membrane. H⁺ then flow back through the membrane by way of ATP synthase, which catalyzes the formation of ATP. The location of the electron transport chain is the inner mitochondrial matrix in eukaryotic cells and cytoplasmic membrane in prokaryotic cells.

Calvin-Benson Cycle



The Calvin-Benson cycle has three stages. In stage 1, the enzyme RuBisCO incorporates carbon dioxide into an organic molecule, 3-PGA. In stage 2, the organic molecule is reduced using electrons supplied by NADPH. In stage 3, RuBP, the molecule that starts the cycle, is regenerated so that the cycle can continue. Only one carbon dioxide molecule is incorporated at a time, so the cycle must be completed three times to produce a single three-carbon GA3P molecule, and six times to produce a six-carbon glucose molecule.

Taxonomy of Clinically Relevant Microorganisms

Bacterial Pathogens

The following tables list the species, and some higher groups, of pathogenic Eubacteria mentioned in the text. The classification of Bacteria, one of the three domains of life, is in constant flux as relationships become clearer through sampling of genetic sequences. Many groups at all taxonomic levels still have an undetermined relationship with other members of the phylogenetic tree of Bacteria. *Bergey's Manual of Systematics of Archaea and Bacteria* maintains a published list and descriptions of prokaryotic species. The tables here follow the taxonomic organization in the *Bergey's Manual Taxonomic Outline*.^[footnote] Bergey's Manual Trust. *Bergey's Manual of Systematics of Archaea and Bacteria, Taxonomic Outline*. 2012. <http://www.bergeys.org/outlines.html>

We have divided the species into tables corresponding to different bacterial phyla. The taxonomic rank of kingdom is not used in prokaryote taxonomy, so the phyla are the subgrouping below domain. Note that many bacterial phyla not represented by these tables. The species and genera are listed only under the class within each phylum. The names given to bacteria are regulated by the International Code of Nomenclature of Bacteria as maintained by the International Committee on Systematics or Prokaryotes.

Phylum Actinobacteria			
Class	Genus	Species	Related Diseases
Actinobacteria	<i>Corynebacterium</i>	<i>diphtheriae</i>	Diphtheria
	<i>Gardnerella</i>	<i>vaginalis</i>	Bacterial vaginosis
	<i>Micrococcus</i>		Opportunistic infections
	<i>Mycobacterium</i>	<i>bovis</i>	Tuberculosis, primarily in cattle
	<i>Mycobacterium</i>	<i>leprae</i>	Hansen's disease
	<i>Mycobacterium</i>	<i>tuberculosis</i>	Tuberculosis
	<i>Propionibacterium</i>	<i>acnes</i>	Acne, blepharitis, endophthalmitis

Phylum Bacteroidetes

Class	Genus	Species	Related Diseases
Bacteroidia	<i>Porphyromonas</i>		Periodontal disease
	<i>Prevotella</i>	<i>intermedia</i>	Periodontal disease

Phylum Chlamydiae

Class	Genus	Species	Related Diseases
Chlamydiae	<i>Chlamydia</i>	<i>psittaci</i>	Psittacosis
	<i>Chlamydia</i>	<i>trachomatis</i>	Sexually transmitted chlamydia

Phylum Firmicutes

Class	Genus	Species	Related Diseases
Bacilli	<i>Bacillus</i>	<i>anthracis</i>	Anthrax
	<i>Bacillus</i>	<i>cereus</i>	Diarrheal and emetic food poisoning
	<i>Listeria</i>	<i>monocytogenes</i>	Listeriosis
	<i>Enterococcus</i>	<i>faecalis</i>	Endocarditis, septicemia, urinary tract infections, meningitis
	<i>Staphylococcus</i>	<i>aureus</i>	Skin infections, sinusitis, food poisoning

Phylum Firmicutes			
Class	Genus	Species	Related Diseases
Clostridia	<i>Staphylococcus</i>	<i>epidermidis</i>	Nosocomial and opportunistic infections
	<i>Staphylococcus</i>	<i>hominis</i>	Opportunistic infections
	<i>Staphylococcus</i>	<i>saprophyticus</i>	Urinary tract infections
	<i>Streptococcus</i>	<i>agalactiae</i>	Postpartum infection, neonatal sepsis
	<i>Streptococcus</i>	<i>mutans</i>	Tooth decay
	<i>Streptococcus</i>	<i>pneumoniae</i>	Pneumonia, many other infections
	<i>Streptococcus</i>	<i>pyogenes</i>	Pharyngitis, scarlet fever, impetigo, necrotizing fasciitis
	<i>Clostridium</i>	<i>botulinum</i>	Botulinum poisoning
Clostridia	<i>Clostridium</i>	<i>difficile</i>	Colitis
	<i>Clostridium</i>	<i>perfringens</i>	Food poisoning, gas gangrene
	<i>Clostridium</i>	<i>tetani</i>	Tetanus

Phylum Fusobacteria			
Class	Genus	Species	Related Diseases
Fusobacteriia	<i>Fusobacterium</i>		Periodontal disease, Lemierre syndrome, skin ulcers
	<i>Streptobacillus</i>	<i>moniliformis</i>	Rat-bite fever

Phylum Proteobacteria

Class	Genus	Species	Related Diseases
Alphaproteobacteria	<i>Anaplasma</i>	<i>phagocytophilum</i>	Human granulocytic anaplasmosis
	<i>Bartonella</i>	<i>henselae</i>	Peliosis hepatitis, bacillary angiomatosis, endocarditis, bacteremia
	<i>Bartonella</i>	<i>quintana</i>	Trench fever
	<i>Brucella</i>	<i>melitensis</i>	Ovine brucellosis
	<i>Ehrlichia</i>	<i>chaffeensis</i>	Human monocytic ehrlichiosis
	<i>Rickettsia</i>	<i>prowazekii</i>	Epidemic typhus
	<i>Rickettsia</i>	<i>rickettsii</i>	Rocky Mountain spotted fever
	<i>Rickettsia</i>	<i>typhi</i>	Murine typhus
Betaproteobacteria	<i>Bordetella</i>	<i>pertussis</i>	Pertussis
	<i>Eikenella</i>		Bite-injury infections
	<i>Neisseria</i>	<i>gonorrhoeae</i>	Gonorrhea
	<i>Neisseria</i>	<i>meningitidis</i>	Meningitis

Phylum Proteobacteria			
Class	Genus	Species	Related Diseases
	<i>Spirillum</i>	<i>minus</i> (<i>alt. minor</i>)	Sodoku (rat-bite fever)
Epsilonproteobacteria	<i>Campylobacter</i>	<i>jejuni</i>	Gastroenteritis, Guillain-Barré syndrome
	<i>Helicobacter</i>	<i>pylori</i>	Gastric ulcers
Gammaproteobacteria	<i>Aeromonas</i>	<i>hydrophila</i>	Dysenteric gastroenteritis
	<i>Coxiella</i>	<i>burnetii</i>	Q fever
	<i>Enterobacter</i>		Urinary and respiratory infections
	<i>Escherichia</i>	<i>coli</i> Strains: shiga toxin-producing (STEC) (e.g., O157:H7) also called enterohemorrhagic <i>E. coli</i> (EHEC) or verocytotoxin-producing <i>E. coli</i> (VTEC)	Foodborne diarrhea outbreaks, hemorrhagic colitis, hemolytic-uremic syndrome
	<i>Escherichia</i>	<i>coli</i> Strain: enterotoxigenic <i>E. coli</i> (ETEC)	Traveler's diarrhea
	<i>Escherichia</i>	<i>coli</i> Strain: enteropathogenic <i>E. coli</i> (EPEC)	Diarrhea, especially in young children

Phylum Proteobacteria			
Class	Genus	Species	Related Diseases
	<i>Escherichia</i>	<i>coli</i> Strain: enteroaggregative <i>E. coli</i> (EAEC)	Diarrheal disease in children and travelers
	<i>Escherichia</i>	<i>coli</i> Strain: diffusely adherent <i>E. coli</i> (DAEC)	Diarrheal disease of children
	<i>Escherichia</i>	<i>coli</i> Strain: enteroinvasive <i>E. coli</i> (EPEC)	Bacillary dysentery, cells invade intestinal epithelial cells
	<i>Francisella</i>	<i>tularensis</i>	Tularemia
	<i>Haemophilus</i>	<i>ducreyi</i>	Chancroid
	<i>Haemophilus</i>	<i>influenzae</i>	Bacteremia, pneumonia, meningitis
	<i>Klebsiella</i>	<i>pneumoniae</i>	Pneumonia, nosocomial infections
	<i>Legionella</i>	<i>pneumophila</i>	Legionnaire's disease
	<i>Moraxella</i>	<i>catarrhalis</i>	Otitis media, bronchitis, sinusitis, laryngitis, pneumonia
	<i>Pasteurella</i>		Pasteurellosis

Phylum Proteobacteria			
Class	Genus	Species	Related Diseases
	<i>Plesiomonas</i>	<i>shigelloides</i>	Gastroenteritis
	<i>Proteus</i>		Opportunistic urinary tract infections
	<i>Pseudomonas</i>	<i>aeruginosa</i>	Opportunistic, nosocomial pneumonia and sepsis
	<i>Salmonella</i>	<i>bongori</i>	Salmonellosis
	<i>Salmonella</i>	<i>enterica</i>	Salmonellosis
	<i>Serratia</i>		Pneumonia, urinary tract infections
	<i>Shigella</i>	<i>boydii</i>	Dysentery
	<i>Shigella</i>	<i>dysenteriae</i>	Dysentery
	<i>Shigella</i>	<i>flexneri</i>	Dysentery
	<i>Shigella</i>	<i>sonnei</i>	Dysentery
	<i>Vibrio</i>	<i>cholerae</i>	Cholera
	<i>Vibrio</i>	<i>parahemolyticus</i>	Seafood gastroenteritis
	<i>Vibrio</i>	<i>vulnificus</i>	Seafood gastroenteritis, necrotizing wound infections, septicemia

Phylum Proteobacteria			
Class	Genus	Species	Related Diseases
	<i>Yersinia</i>	<i>enterocolitica</i>	Yersiniosis
	<i>Yersinia</i>	<i>pestis</i>	Plague
	<i>Yersinia</i>	<i>pseudotuberculosis</i>	Far East scarlet-like fever

Phylum Spirochaetes			
Class	Genus	Species	Related Diseases
Spirochaetia	<i>Borrelia</i>	<i>burgdorferi</i>	Lyme disease
	<i>Borrelia</i>	<i>hermsii</i>	Tick-borne relapsing fever
	<i>Borrelia</i>	<i>recurrentis</i>	Louse-borne relapsing fever
	<i>Leptospira</i>	<i>interrogans</i>	Leptospirosis
	<i>Treponema</i>	<i>pallidum</i>	Syphilis, bejel, pinta, yaws

Phylum Tenericutes			
Class	Genus	Species	Related Diseases
Mollicutes	<i>Mycoplasma</i>	<i>genitalium</i>	Urethritis, cervicitis

Phylum Tenericutes			
Class	Genus	Species	Related Diseases
	<i>Mycoplasma</i>	<i>hominis</i>	Pelvic inflammatory disease, bacterial vaginosis
	<i>Mycoplasma</i>	<i>pneumoniae</i>	<i>Mycoplasma</i> pneumonia
	<i>Ureaplasma</i>	<i>urealyticum</i>	Urethritis, fetal infections

Viral Pathogens

There are several classification systems for viruses. The International Committee on Taxonomy of Viruses (ICTV) is the international scientific body responsible for the rules of viral classification. The ICTV system used here groups viruses based on genetic similarity and presumed monophyly. The viral classification system is separate from the classification system for cellular organisms. The ICTV system groups viruses within seven orders, which contain related families. There is, presently, a large number of unassigned families with unknown affinities to the seven orders. Three of these orders infect only Eubacteria, Archaea, or plants and do not appear in this table. Some families may be divided into subfamilies. There are also many unassigned genera. Like all taxonomies, viral taxonomy is in constant flux. The latest complete species list and classification can be obtained on the ICTV website.[\[footnote\]](#)

International Committee on Taxonomy of Viruses. “ICTV Master Species List.” http://talk.ictvonline.org/files/ictv_documents/m/msl/default.aspx

Viral Pathogens					
Order	Family	Sub-family	Genus	Species	Related diseases
<i>Herpesvirales</i>	<i>Herpesviridae</i>	<i>Betaherpesvirinae</i>	<i>Human cytomegalovirus group</i>	<i>Human herpesvirus 5</i>	Cytomegalovirus hepatitis and other infections in immunocompromised people
		<i>Gammaherpesvirinae</i>	<i>Lymphocryptovirus</i>	<i>Human herpesvirus 4 (HHV-4; Epstein-Barr virus)</i>	Infectious mononucleosis
		<i>Alphaherpesvirinae</i>	<i>Simplexvirus</i>	<i>Human herpesvirus 1, human herpesvirus 2</i>	Herpes simplex virus 1, herpes simplex virus 2
			<i>Varicellovirus</i>	<i>Human herpesvirus 3</i>	Chicken pox, shingles
<i>Mononegavirales</i>	<i>Filoviridae</i>		<i>Ebolavirus</i>	<i>Zaire ebolavirus (EBOV)</i>	Ebola
			<i>Marburgvirus</i>	<i>Marburg marburgvirus (MARV)</i>	Marburg virus disease
	<i>Rhabdoviridae</i>		<i>Lyssavirus</i>	<i>Rabies virus</i>	Rabies
	<i>Paramyxoviridae</i>	<i>Pneumovirinae</i>	<i>Pneumovirus</i>	<i>Human respiratory syncytial virus</i>	Lower respiratory tract infection
		<i>Paramyxovirinae</i>	<i>Morbillivirus</i>	<i>Measles virus</i>	Measles (rubeola)
<i>Nidovirales</i>	<i>Coronaviridae</i>	<i>Coronavirinae</i>	<i>Coronavirus</i>		Common cold, pneumonia, SARS
<i>Picornavirales</i>	<i>Picornaviridae</i>		<i>Hepatovirus</i>	<i>Hepatitis A virus</i>	Hepatitis A
			<i>Enterovirus</i>	<i>Enterovirus C</i>	Polio
				<i>Rhinovirus A</i>	Common cold
				<i>Rhinovirus B</i>	Common cold
				<i>Rhinovirus C</i>	Common cold
<i>Unassigned</i>	<i>Adenovirus</i>		<i>Mastadenovirus</i>		Respiratory and other infections
	<i>Arenaviridae</i>		<i>Mammarenavirus</i>	<i>Lassa mammarenavirus</i>	Lassa fever
	<i>Astroviridae</i>				Gastroenteritis
	<i>Bunyaviridae</i>		<i>Hantavirus</i>	Several species	Hantavirus hemorrhagic fever with renal syndrome (HFRS), hantavirus pulmonary syndrome (HPS)
			<i>Nairovirus</i>	<i>Crimean-Congo hemorrhagic fever virus (CCHF)</i>	Crimean-Congo hemorrhagic fever
	<i>Caliciviridae</i>		<i>Norovirus</i>	<i>Norwalk virus</i>	Gastroenteritis

Viral Pathogens (continued)					
Order	Family	Sub-family	Genus	Species	Related diseases
Unassigned	<i>Flaviviridae</i>		<i>Flavivirus</i>	<i>Dengue virus</i>	Dengue fever
				<i>Yellow fever virus</i>	Yellow fever
			<i>Hepacivirus</i>	<i>Hepatitis C virus</i>	Hepatitis C
	<i>Hepadnaviridae</i>		<i>Orthohepadnavirus</i>	<i>Hepatitis B virus</i>	Hepatitis B
	<i>Hepeviridae</i>		<i>Orthohepevirus</i>	<i>Hepatitis E virus</i>	Hepatitis E
	<i>Orthomyxoviridae</i>		<i>Influenzavirus A</i>	<i>Influenza A virus</i>	Pandemic flu
			<i>Influenzavirus B</i>	<i>Influenza B virus</i>	Flu
			<i>Influenzavirus C</i>	<i>Influenza C virus</i>	Flu
	<i>Papillomaviridae</i>		<i>Alphapapillomavirus</i>	<i>Human papillomavirus</i>	Skin warts
	<i>Parvoviridae</i>	<i>Parvovirinae</i>	<i>Erythroparvovirus</i>	<i>Human parvovirus B19</i>	Fifth disease (erythema infectosum)
	<i>Poxviridae</i>	<i>Chordopoxvirinae</i>	<i>Orthopoxvirus</i>	<i>Variola virus</i>	Variola major, Variola minor (smallpox)
				<i>Vaccinia virus</i>	Cowpox
	<i>Reoviridae</i>	<i>Sedoreovirinae</i>	<i>Rotavirus</i>	Eight species	Gastroenteritis
	<i>Retroviridae</i>	<i>Orthoretrovirinae</i>	<i>Lentivirus</i>	<i>Human immunodeficiency virus</i>	AIDS
	<i>Togaviridae</i>		<i>Alphavirus</i>	<i>Chikungunya virus (CHIKV)</i>	Chikungunya
			<i>Rubivirus</i>	<i>Rubella virus</i>	Rubella (German measles)
	Unassigned		<i>Deltavirus</i>	<i>Hepatitis D virus</i>	Hepatitis D

Fungal Pathogens

The Fungi are one of the kingdoms of the domain Eukarya. Fungi are most closely related to the animals and a few other small groups and more distantly related to the plants and other groups that formerly were categorized as protist. At present, the Fungi are divided into seven phyla (or divisions, a hold over from when fungi were studied with plants), but there are uncertainties about some relationships.[\[footnote\]](#) Many groups of fungi, particularly those that were formerly classified in the phylum Zygomycota, which was not monophyletic, have uncertain relationships to the other fungi. The one species listed in this table that falls into this category is *Rhizopus arrhizus*. Fungal names are governed by the International Code of Nomenclature for Algae, Fungi, and Plants,[\[footnote\]](#) but the International Commission on the Taxonomy of Fungi (ICTF) also promotes taxonomic work on fungi. One activity of the ICTF is publicizing name changes for medically and otherwise important fungal species. Many species that formerly had two names (one for the sexual form and one for the asexual form) are now being brought together under one name.

D. S. Hibbett et al. “A Higher-level Phylogenetic Classification of the Fungi.” *Mycological Research* 111 no. 5 (2007):509–547.

J. McNeill et al. *International Code of Nomenclature for Algae, Fungi, and Plants (Melbourne Code)*. Oberreifenerg, Germany. Koeltz Scientific Books; 2012. <http://www.iapt-taxon.org/nomen/main.php?>

Fungal Pathogens			
Division	Genus	Species	Related Diseases
Ascomycota	<i>Aspergillus</i>	<i>flavus</i>	Opportunistic aspergillosis
	<i>Aspergillus</i>	<i>fumigatus</i>	Opportunistic aspergillosis
	<i>Blastomyces</i>	<i>dermatitidis</i>	Blastomycosis
	<i>Candida</i>	<i>albicans</i>	Thrush (candidiasis)
	<i>Coccidioides</i>	<i>immitis</i>	Valley fever (coccidioidomycosis)
	<i>Epidermophyton</i>		Tinea corporis (ringworm), tinea cruris (jock itch), tinea pedis (athlete's foot), tinea unguium (onychomycosis)
	<i>Histoplasma</i>	<i>capsulatum</i>	Histoplasmosis
	<i>Microsporum</i>		Tinea capitis (ringworm), tinea corpus (ringworm), other dermatophytoses
	<i>Pneumocystis</i>	<i>jirovecii</i>	Opportunistic pneumonia
	<i>Sporothrix</i>	<i>schenckii</i>	Sporotrichosis (rose-handler's disease)

Fungal Pathogens			
Division	Genus	Species	Related Diseases
	<i>Trichophyton</i>	<i>mentagrophytes</i> var. <i>interdigitale</i>	Tinea barbae (barber's itch), dermatophytoses
	<i>Trichophyton</i>	<i>ruberum</i>	Tinea corporis (ringworm), tinea cruris (jock itch), tinea pedis (athlete's foot), tinea unguium (onychomycosis)
Basidiomycota	<i>Cryptococcus</i>	<i>neoformans</i>	Opportunistic cryptococcosis, fungal meningitis, encephalitis
	<i>Malassezia</i>		Dandruff, tinea versicolor
uncertain	<i>Rhizopus</i>	<i>arrhizus</i>	Mucormycosis

Protozoan Pathogens

The relationships among the organisms (and thus their taxonomy) previously grouped under the name Protists are better understood than they were two or three decades ago, but this is still a work in progress. In 2005, the Eukarya were divided into six supergroups.[\[footnote\]](#) The latest high-level classification combined two of the previous supergroups to produce a system comprising five supergroups.[\[footnote\]](#) This classification was developed for the Society of Protozoologists, but it is not the only suggested approach. One of the five supergroups includes the animals, fungi, and some smaller protist groups. Another contains green plants and three algal groups. The other three supergroups (listed in the three tables below) contain the other protists, many of them which cause disease. In addition, there is a large number of protist groups whose relationships are not understood. In the three supergroups represented here we have indicated the phyla to which the listed pathogens belong.

S.M. Adl et al. “The New Higher Level Classification of Eukaryotes with Emphasis on the Taxonomy of Protists.” *Journal of Eukaryotic Microbiology* 52 no. 5 (2005):399–451.

S.M. Adl et al. “The Revised Classification of Eukaryotes.” *Journal of Eukaryotic Microbiology* 59 no. 5 (2012):429–514.

Supergroup Amoebozoa			
Phylum	Genus	Species	Related Diseases
Amoebozoa	<i>Acanthamoeba</i>		Granulomatous amoebic encephalitis, acanthamoebic keratitis
	<i>Entamoeba</i>	<i>histolytica</i>	Enterobiasis

Supergroup SAR (Stramenopiles, Alveolata, Rhizaria)			
Phylum	Genus	Species	Related Diseases
Apicomplexa	<i>Babesia</i>		Babesiosis
	<i>Cryptosporidium</i>	<i>hominis</i>	Cryptosporidiosis
	<i>Cryptosporidium</i>	<i>parvum</i>	Cryptosporidiosis
	<i>Cyclospora</i>	<i>cayetanensis</i>	Gastroenteritis
	<i>Plasmodium</i>	<i>falciparum</i>	Malaria
	<i>Plasmodium</i>	<i>malariae</i>	“Benign” or “quartan” (3-day recurrent fever) malaria
	<i>Plasmodium</i>	<i>ovale</i>	“Tertian” (2-day recurrent fever) malaria
	<i>Plasmodium</i>	<i>vivax</i>	“Benign” “tertian” (2-day recurrent fever) malaria
	<i>Plasmodium</i>	<i>knowlesi</i>	Primate malaria capable of zoonosis, quotidian fever
	<i>Toxoplasma</i>	<i>gondii</i>	Toxoplasmosis

Supergroup Excavata

Phylum	Genus	Species	Related Diseases
Metamonada	<i>Giardia</i>	<i>lamblia</i>	Giardiasis
	<i>Trichomonas</i>	<i>vaginalis</i>	Trichomoniasis
Euglenozoa	<i>Leishmania</i>	<i>braziliensis</i>	Leishmaniasis
	<i>Leishmania</i>	<i>donovani</i>	Leishmaniasis
	<i>Leishmania</i>	<i>tropica</i>	Cutaneous leishmaniasis
	<i>Trypanosoma</i>	<i>brucei</i>	African sleeping sickness (African trypanosomiasis)
	<i>Trypanosoma</i>	<i>cruzi</i>	Chagas disease
Percolozoa	<i>Naegleria</i>	<i>fowleri</i>	Primary amoebic meningoencephalitis (naegleriasis)

Parasitic Helminths

The taxonomy of parasitic worms, all of which belong to the kingdom Animalia still contains many uncertainties. The pathogenic species are found in two phyla: the Nematoda, or roundworms, and the Platyhelminthes, or flat worms. The Nematoda is tentatively divided into two classes[[footnote](#)], one of which, Chromadorea, probably contains unrelated groups. The parasitic flatworms are contained within three classes of flatworm, of which two are important to humans, the trematodes and the cestodes.

National Center for Biotechnology Information. “Taxonomy Browser: Nematoda.”
<http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=6231>

Phylum Nematoda

Class	Genus	Species	Related Diseases
Chromadorea	<i>Ancylostoma</i>	<i>caninum</i>	Dog hookworm infection

Phylum Nematoda			
Class	Genus	Species	Related Diseases
	<i>Ancylostoma</i>	<i>duodenale</i>	Old World hookworm infection
	<i>Ascaris</i>	<i>lumbricoides</i>	Ascariasis
	<i>Enterobius</i>	<i>vermicularis</i>	Enterobiasis (pin worm)
	<i>Loa</i>	<i>loa</i>	Loa loa filariasis (eye worm)
	<i>Necator</i>	<i>americanus</i>	Necatoriasis (New World hookworm infection)
	<i>Strongyloides</i>	<i>stercoralis</i>	Strongyloidiasis
Enoplea	<i>Trichinella</i>	<i>spiralis</i>	Trichinosis
	<i>Trichuris</i>	<i>trichiura</i>	Trichuriasis (whip worm infection)

Phylum Platyhelminthes			
Class	Genus	Species	Related Diseases
Trematoda	<i>Clonorchis</i>	<i>sinensis</i>	Chinese liver fluke
	<i>Fasciolopsis</i>	<i>buski</i>	Fasciolopsiasis
	<i>Fasciola</i>	<i>gigantica</i>	Fascioliasis
	<i>Fasciola</i>	<i>hepatica</i>	Fascioliasis
	<i>Opisthorchis</i>	<i>felineus</i>	Opisthorchiasis
	<i>Opisthorchis</i>	<i>viverrini</i>	Opisthorchiasis

Phylum Platyhelminthes			
Class	Genus	Species	Related Diseases
Cestoda	<i>Schistosoma</i>	<i>haematobium</i>	Urinary schistosomiasis
	<i>Schistosoma</i>	<i>japonicum</i>	Schistosomiasis
	<i>Schistosoma</i>	<i>mansoni</i>	Intestinal schistosomiasis
	<i>Diphyllobothrium</i>	<i>latum</i>	Diphyllobothriosis
	<i>Echinococcus</i>	<i>granulosus</i>	Hydatid cysts (cystic echinococcosis)
	<i>Echinococcus</i>	<i>multilocularis</i>	Echinococcosis
Nematoda	<i>Taenia</i>	<i>asiatica</i>	Intestinal taeniasis
	<i>Taenia</i>	<i>saginata</i>	Intestinal taeniasis
	<i>Taenia</i>	<i>solium</i>	Intestinal taeniasis, cysticercosis

Glossary

- **454 sequencing (pyrosequencing)** a next generation sequencing technique in which fragmented DNA has DNA adapters attached, is amplified by PCR, is attached to a bead, and then placed into a well with sequencing reagents, and the flash of light produced by the release of pyrophosphate on addition of a nucleotide is monitored
- **5' cap** methylguanosine nucleotide added to 5' end of a eukaryotic primary transcript
- **70S ribosome** a ribosome composed of 50S and 30S subunits
- **80S ribosome** cytoplasmic eukaryotic ribosome composed of 60S and 40S subunits

A

- **α -helix** secondary structure consisting of a helix stabilized by hydrogen bonds between nearby amino acid residues in a polypeptide
- **A (aminoacyl) site** functional site of an intact ribosome that binds incoming charged aminoacyl tRNAs
- **A-B exotoxin** class of exotoxin that contains A subunits, which enter the cell and disrupt cellular activities, and B subunits, which bind to host cell receptors
- **ABO blood group system** set of glycoprotein antigens found on the surface of red blood cells; the presence or absence of specific carbohydrates determining blood type
- **absorbance** when a molecule captures energy from a photon and vibrates or stretches, using the energy
- **Acanthamoeba keratitis** a condition characterized by damage to the cornea and possible blindness caused by parasitic infection of the protozoan *Acanthamoeba*
- **acellular** not made of cells
- **acid-fast stain** a stain that differentiates cells that have waxy mycolic acids in their gram-positive cell walls

- **acidic dye** a chromophore with a negative charge that attaches to positively charged structures
- **acidophile** organism that grows optimally at a pH near 3.0
- **acne** a skin disease in which hair follicles or pores become clogged, leading to the formation of comedones and infected lesions
- **acquired immunodeficiency syndrome (AIDS)** disease caused by HIV, characterized by opportunistic infections and rare cancers
- **actin** a protein that polymerizes to form microfilaments
- **activation energy** energy needed to form or break chemical bonds and convert a reactant or reactants to a product or products
- **activator** protein that increases the transcription of a gene in response to an external stimulus
- **active carrier** an infected individual who can transmit the pathogen to others regardless of whether symptoms are currently present
- **active immunity** stimulation of one's own adaptive immune responses
- **active site** location within an enzyme where substrate(s) bind
- **acute disease** disease of a relatively short duration that develops and progresses in a predictable pattern
- **acute glomerulonephritis** inflammation of the glomeruli of the kidney, probably resulting from deposition of immune complexes and an autoimmune response caused by self-antigen mimicry by a pathogen
- **acute necrotizing ulcerative gingivitis** a severe form of gingivitis, also called trench mouth
- **acute otitis media** inflammatory disease of the middle ear resulting from a microbial infection
- **acute rheumatic fever** sequela of streptococcal pharyngitis; comorbidities include arthritis and carditis
- **acute-phase proteins** antimicrobial molecules produced by liver cells in response to pathogen-induced stimulation events
- **acyclovir** antiviral guanosine analog; inhibits DNA replication
- **adaptive immunity** third-line defense characterized by specificity and memory
- **Addison disease** autoimmune disease affecting adrenal gland function
- **adenine** purine nitrogenous base found in nucleotides
- **adenosine diphosphate (ADP)** nucleotide derivative and relative of ATP containing only one high-energy phosphate bond

- **adenosine monophosphate (AMP)** adenine molecule bonded to a ribose molecule and to a single phosphate group, having no high-energy phosphate bonds
- **adenosine triphosphate (ATP)** energy currency of the cell; a nucleotide derivative that safely stores chemical energy in its two high-energy phosphate bonds
- **adhesins** molecules on the surface of pathogens that promote colonization of host tissue
- **adhesion** the capability of microbes to attach to host cells
- **aerobic respiration** use of an oxygen molecule as the final electron acceptor of the electron transport system
- **aerotolerant anaerobe** organism that does not use oxygen but tolerates its presence
- **affinity maturation** function of the immune system by which B cells, upon re-exposure to antigen, are selected to produce higher affinity antibodies
- **affinity** measure of how tightly an antibody-binding site binds to its epitope
- **aflatoxin** chemical produced by the fungus *Aspergillus flavus*; both a toxin and the most potent known natural carcinogen
- **African sleeping sickness** see *human African trypanosomiasis*
- **agarose gel electrophoresis** a method for separating populations of DNA molecules of varying sizes by differential migration rates caused by a voltage gradient through a horizontal gel matrix
- **agglutination** binding of different pathogen cells by Fab regions of the same antibody to aggregate and enhance elimination from body
- **agranulocytes** leukocytes that lack granules in the cytoplasm
- **alarmone** small intracellular derivative of a nucleotide that signals a global bacterial response (i.e., activating a regulon of operons) to an environmental stress
- **albendazole** antihelminthic drug of the benzimidazole class that binds to helminthic β -tubulin, preventing microtubule formation
- **algae** (singular: alga) any of various unicellular and multicellular photosynthetic eukaryotic organisms; distinguished from plants by their lack of vascular tissues and organs
- **alkaliphile** organism that grows optimally at pH above 9.0

- **alkylating agent** type of strong disinfecting chemical that acts by replacing a hydrogen atom within a molecule with an alkyl group, thereby inactivating enzymes and nucleic acids
- **allergen** antigen capable of inducing type I hypersensitivity reaction
- **allergy** hypersensitivity response to an allergen
- **allograft** transplanted tissue from an individual of the same species that is genetically different from the recipient
- **allosteric activator** molecule that binds to an enzyme's allosteric site, increasing the affinity of the enzyme's active site for the substrate(s)
- **allosteric site** location within an enzyme, other than the active site, to which molecules can bind, regulating enzyme activity
- **allylamines** class of antifungal drugs that inhibit ergosterol biosynthesis at an early point in the pathway
- **Alphaproteobacteria** class of Proteobacteria that are all oligotrophs
- **alveoli** cul-de-sacs or small air pockets within the lung that facilitate gas exchange
- **amantadine** antiviral drug that targets the influenza virus by preventing viral escape from endosomes upon host cell uptake, thus preventing viral RNA release and subsequent viral replication
- **amensalism** type of symbiosis in which one population harms the other but remains unaffected itself
- **Ames test** method that uses auxotrophic bacteria to detect mutations resulting from exposure to potentially mutagenic chemical compounds
- **amino acid** a molecule consisting of a hydrogen atom, a carboxyl group, and an amine group bonded to the same carbon. The group bonded to the carbon varies and is represented by an *R* in the structural formula
- **aminoacyl-tRNA synthetase** enzyme that binds to a tRNA molecule and catalyzes the addition of the correct amino acid to the tRNA
- **aminoglycosides** protein synthesis inhibitors that bind to the 30S subunit and interfere with the ribosome's proofreading ability, leading to the generation of faulty proteins that insert into and disrupt the bacterial cytoplasmic membrane
- **amoebiasis** intestinal infection caused by *Entamoeba histolytica*
- **amoebic dysentery** severe form of intestinal infection caused by *Entamoeba histolytica*, characterized by severe diarrhea with blood and mucus

- **amphipathic** a molecule containing both polar and nonpolar parts
- **amphitrichous** having two flagella or tufts of multiple flagella, with one flagellum or tuft located at each end of the bacterial cell
- **amphotericin B** antifungal drug of the polyene class that is used to treat several systemic fungal infections
- **amplitude** the height of a wave
- **anabolism** chemical reactions that convert simpler molecules into more complex ones
- **anaerobe chamber** closed compartment used to handle and grow obligate anaerobic cultures
- **anaerobe jar** container devoid of oxygen used to grow obligate anaerobes
- **anaerobic respiration** use of a non-oxygen inorganic molecule, like CO₂, nitrate, nitrite, oxidized iron, or sulfate, as the final electron acceptor at the end of the electron transport system
- **analytical epidemiology** study of disease outbreaks to establish associations between an agent and a disease state through observational studies comparing groups of individuals
- **anaphylactic shock** another term for anaphylaxis
- **anaphylaxis** systemic and potentially life-threatening type I hypersensitivity reaction
- **anergy** peripheral tolerance mechanism that prevents self-reactive T cells from being activated by self-antigens through lack of co-stimulation
- **annealing** formation of hydrogen bonds between the nucleotide base pairs of two single-stranded complementary nucleic acid sequences
- **anoxygenic photosynthesis** type of photosynthesis found in many photosynthetic bacteria, including the purple and green bacteria, where an electron donor other than H₂O is used to replace an electron lost by a reaction center pigment, resulting no oxygen production
- **anthrax** a disease caused by *Bacillus anthracis*; the cutaneous form causes a skin lesion to develop; gastrointestinal and inhalation anthrax have high mortality rates
- **antibiogram** compilation of the antimicrobial susceptibilities recorded for local bacterial strains, which is useful for monitoring local trends in antimicrobial resistance and aiding the prescription of appropriate empiric antibacterial therapy

- **antibiotic-associated diarrhea** diarrhea that develops after antibiotic treatment as a result of disruption to the normal microbiota; *C. difficile* is a particularly serious example
- **antibody screen** test to make sure that a potential blood recipient has not produced antibodies to antigens other than the ABO and Rh antigens
- **antibody** Y-shaped glycoprotein molecule produced by B cells that binds to specific epitopes on an antigen
- **antibody-dependent cell-mediated cytotoxicity (ADCC)** mechanism by which large pathogens are marked for destruction by specific antibodies and then killed by secretion of cytotoxins by natural killer cells, macrophages, or eosinophils
- **anticodon** three-nucleotide sequence of a mature tRNA that interacts with an mRNA codon through complementary base pairing
- **antigen (also, immunogen)** a molecule that stimulates an adaptive immune response
- **antigenic** able to stimulate an adaptive immune response
- **antigenic drift** form of slight antigenic variation that occurs because of point mutations in the genes that encode surface proteins
- **antigenic shift** form of major antigenic variation that occurs because of gene reassortment
- **antigenic variation** changing of surface antigens (carbohydrates or proteins) such that they are no longer recognized by the host's immune system
- **antigen-presenting cells (APC)** macrophages, dendritic cells, and B cells that process and present foreign pathogen antigens for the purpose of activating T cells and adaptive immune defenses
- **antimetabolites** compounds that are competitive inhibitors for bacterial metabolic enzymes
- **antimicrobial drugs** chemical compounds, including naturally produced drugs, semisynthetic derivatives, and synthetic compounds, that target specific microbial structures and enzymes, killing specific microbes or inhibiting their growth
- **antimicrobial peptides (AMPs)** class of nonspecific, cell-derived chemical mediators with broad-spectrum antimicrobial properties
- **antiparallel** two strands of DNA helix oriented in opposite directions; one strand is oriented in the 5' to 3' direction, while the other is

oriented in the 3' to 5' direction

- **antisense RNA** small noncoding RNA molecules that inhibit gene expression by binding to mRNA transcripts via complementary base pairing
- **antisense strand** transcription template strand of DNA; the strand that is transcribed for gene expression
- **antiseptis** protocol that removes potential pathogens from living tissue
- **antiseptic** antimicrobial chemical that can be used safely on living tissue
- **antiserum** serum obtained from an animal containing antibodies against a particular antigen that was artificially introduced to the animal
- **apoenzyme** enzyme without its cofactor or coenzyme
- **apoptosis** programmed and organized cell death without lysis of the cell
- **arachnoid mater** middle membrane surrounding the brain that produces cerebrospinal fluid
- **arboviral encephalitis** infection by an arthropod-borne virus that results in an inflammation of the brain
- **arbovirus** any of a variety of viruses that are transmitted by arthropod vectors
- **archaea** any of various unicellular prokaryotic microorganisms, typically having cell walls containing pseudopeptidoglycan
- **Archaea** domain of life separate from the domains Bacteria and Eukarya
- **artemisinin** antiprotozoan and antifungal drug effective against malaria that is thought to increase intracellular levels of reactive oxygen species in target microbes
- **artery** large, thick-walled vessel that carries blood from the heart to the body tissues
- **Arthus reaction** localized type III hypersensitivity
- **artificial active immunity** immunity acquired through exposure to pathogens and pathogen antigens through a method other than natural infection
- **artificial passive immunity** transfer of antibodies produced by a donor to another individual for the purpose of preventing or treating disease

- **ascariasis** soil-transmitted intestinal infection caused by the large nematode roundworm *Ascaris lumbricoides*
- **ascocarps** cup-shaped fruiting bodies of an ascomycete fungus
- **ascospore** asexual spore produced by ascomycete fungi
- **ascus** structure of ascomycete fungi containing spores
- **asepsis** sterile state resulting from proper use of microbial control protocols
- **aseptic technique** method or protocol designed to prevent microbial contamination of sterile objects, locations, or tissues
- **aspergillosis** fungal infection caused by the mold *Aspergillus*; immunocompromised patients are primarily at risk
- **asymptomatic carrier** an infected individual who exhibits no signs or symptoms of disease yet is capable of transmitting the pathogen to others
- **asymptomatic** not exhibiting any symptoms of disease
- **atomic force microscope** a scanning probe microscope that uses a thin probe that is passed just above the specimen to measure forces between the atoms and the probe
- **ATP synthase** integral membrane protein that harnesses the energy of the proton motive force by allowing hydrogen ions to diffuse down their electrochemical gradient, causing components of this protein to spin, making ATP from ADP and P_i
- **attachment** binding of phage or virus to host cell receptors
- **attenuation** regulatory system of prokaryotes whereby secondary stem-loop structures formed within the 5' end of an mRNA being transcribed determine both if transcription to complete the synthesis of this mRNA will occur and if this mRNA will be used for translation
- **autoclave** specialized device for the moist-heat sterilization of materials through the application of pressure to steam, allowing the steam to reach temperatures above the boiling point of water
- **autocrine function** refers to a cytokine signal released from a cell to a receptor on its own surface
- **autograft** tissue transplanted from a location on an individual to a different location on the same individual
- **autoimmune disease** loss of tolerance to self, resulting in immune-mediated destruction of self cells and tissues

- **autoinducer** signaling molecule produced by a bacterial cell that can modify the activity of surrounding cells; associated with quorum sensing
- **autoradiography** the method of producing a photographic image from radioactive decay; in molecular genetics the method allows the visualization of radioactively-labeled DNA probes that have hybridized to a nucleic acid sample
- **autotroph** organism that converts inorganic carbon dioxide into organic carbon
- **auxotroph** nutritional mutant with a loss-of-function mutation in a gene encoding the biosynthesis of a specific nutrient such as an amino acid
- **avidity** strength of the sum of the interactions between an antibody and antigen
- **axon** long projection of a neuron along which an electrochemical signal is transmitted
- **azithromycin** semisynthetic macrolide with increased spectrum of activity, decreased toxicity, and increased half-life compared with erythromycin

B

- **β -lactamases** bacterially produced enzymes that cleave the β -lactam ring of susceptible β -lactam antimicrobials, rendering them inactive and conferring resistance
- **β -lactams** group of antimicrobials that inhibit cell wall synthesis; includes the penicillins, cephalosporins, carbapenems, and monobactams; inhibits the transpeptidase cross-linking activity of penicillin-binding proteins
- **β -oxidation** process of fatty acid degradation that sequentially removes two-carbon acetyl groups, producing NADH and FADH₂, on entry into the Krebs cycle
- **β -pleated sheet** secondary structure consisting of pleats formed by hydrogen bonds between localized segments of amino acid residues on the backbone of the polypeptide chain

- **B-cell receptors (BCRs)** membrane-bound IgD and IgM antibody that bind specific antigen epitopes with Fab antigen-binding region
- **B lymphocyte** antibody-producing cells of humoral immunity; B cell
- **babesiosis** tickborne protozoan infection caused by *Babesia* spp. and characterized by malaise, fatigue, fever, headache, myalgia, and joint pain
- **bacillary dysentery** gastrointestinal illness caused by *Shigella* bacteria, also called shigellosis
- **bacillus** (bacilli) rod-shaped prokaryotic cell
- **bacitracin** group of structurally similar peptides that block the movement of peptidoglycan precursors across the cell membrane, inhibiting peptidoglycan synthesis
- **bacteremia** condition marked by the presence of bacteria in the blood
- **bacteria** (singular: bacterium) any of various unicellular prokaryotic microorganisms typically (but not always) having cell walls that contain peptidoglycan
- **bacterial lawn** layer of confluent bacterial growth on an agar plate
- **bacterial meningitis** bacterial infection that results in an inflammation of the meninges
- **bacterial vaginosis** a condition caused by an overgrowth of bacteria in the vagina that may or may not cause symptoms
- **bactericidal** irreversible inhibition of a microbe's ability to divide
- **bactericide** chemical or physical treatment that kills bacteria
- **bacteriochlorophylls** green, purple, or blue pigments of bacteria; they are similar to chlorophyll of plants
- **bacteriology** the study of bacteria
- **bacteriophage** virus that infects bacteria
- **bacteriostatic** having the ability to inhibit bacterial growth, generally by means of chemical or physical treatment; reversible inhibition of a microbe's ability to divide
- **barophile** organism that grows under high atmospheric pressure
- **basal body** component of eukaryotic flagellum or cilium composed of nine microtubule triplets and attaches the flagellum or cilium to the cell
- **base sequence** identity of the specific nucleotides present in a nucleic acid strand and their order within the strand

- **basic dye** a chromophore with a positive charge that attaches to negatively charged structures
- **basidia (basidium, sing.)** small club-shaped structures of basidiomycete fungi where basidiospores are produced
- **basidiocarps** fruiting bodies of basidiomycete fungi
- **basidiospores** spores produced sexually via budding in basidiomycete fungi
- **basophils** leukocytes with granules containing histamine and other chemicals that facilitate allergic responses and inflammation when released
- **benzimidazoles** class of antihelminthic drugs that bind to helminthic β -tubulin, preventing microtubule formation
- **Betaproteobacteria** class of Proteobacteria that are all eutrophs
- **binary fission** predominant form of bacterial reproduction in which one cell divides into two daughter cells of equal size, which separate, each offspring receiving a complete copy of the parental genome
- **binocular** having two eyepieces
- **binomial nomenclature** a universal convention for the scientific naming of organisms using Latinized names for genus and species
- **biofilm** complex ecosystem of bacteria embedded in a matrix
- **biogeochemical cycle** recycling of inorganic matter between living organisms and their nonliving environment
- **bioinformatics** the analysis of large amounts of information required for interpretation of these data
- **biological transmission** movement of a pathogen between hosts facilitated by a biological vector in which the pathogen grows and reproduces
- **biological vector** an animal (typically an arthropod) that is infected with a pathogen and is capable of transmitting the pathogen from one host to another
- **biomarker** a protein expressed by a cell or tissue that is indicative of disease
- **biomolecule** a molecule that is part of living matter
- **bioremediation** use of microbes to remove xenobiotics or environmental pollutants from a contaminated site
- **biosynthesis** replication of viral genome and other protein components

- **biotechnology** the science of using living systems to benefit humankind
- **bisbiguanide** type of chemical compound with antiseptic properties; disrupts cell membranes at low concentrations and causes congealing of intracellular contents at high concentrations
- **blastomycosis** fungal disease associated with infections by *Blastomyces dermatitidis*; can cause disfiguring scarring of the hands and other extremities
- **blepharitis** inflammation of the eyelids
- **blocking antibodies** antigen-specific antibodies (usually of the IgG type) produced via desensitization therapy
- **blood-brain barrier** tight cell junctions of the endothelia lining the blood vessels that serve the central nervous system, preventing passage of microbes from the bloodstream into the brain and cerebrospinal fluid
- **blue-white screening** a technique commonly used for identifying transformed bacterial cells containing recombinant plasmids using *lacZ*-encoding plasmid vectors
- **blunt ends** ends of DNA molecules lacking single-stranded complementary overhangs that are produced when some restriction enzymes cut DNA
- **botulism** form of flaccid paralysis caused by the ingestion of a neurotoxin produced by *Clostridium botulinum*
- **bradykinin** activated form of a proinflammatory molecule induced in the presence of invader microbes; opens gaps between cells in blood vessels, allowing fluid and cells to leak into surrounding tissue
- **bridge reaction** reaction linking glycolysis to the Krebs cycle during which each pyruvate is decarboxylated and oxidized (forming NADH), and the resulting two-carbon acetyl group is attached to a large carrier called coenzyme A, resulting in the formation of acetyl-CoA and CO; also called the *transition reaction*
- **brightfield microscope** a compound light microscope with two lenses; it produces a dark image on a bright background
- **broad-spectrum antimicrobial** drug that targets many different types of microbes
- **bronchi** major air passages leading to the lungs after bifurcating at the windpipe

- **bronchioles** smaller air passages within the lung that are formed as the bronchi become further subdivided
- **bronchitis** inflammation of the bronchi
- **brucellosis** zoonotic disease caused by bacteria of the genus *Brucella* that results in undulant fever
- **bubo** swollen, inflamed lymph node that forms as a result of a microbial infection
- **bubonic plague** most common form of plague in humans, marked by the presence of swollen lymph nodes (buboës)
- **budding** unequal reproductive division in which a smaller cell detaches from the parent cell
- **budding yeasts** yeasts that divide by budding off of daughter cells
- **Burkitt lymphoma** disease characterized by rapidly growing solid tumor; caused by Epstein-Barr virus (HHV-4)
- **burst** release of new virions by a lysed host cell infected by a virus
- **burst size** the number of virions released from a host cell when it is lysed because of a viral infection

C

- **Calvin-Benson cycle** most common CO₂ fixation pathway in most photoautotrophs; involves light-independent reactions of photosynthesis that occur in the cytoplasm of photosynthetic bacteria and in the stroma of eukaryotic chloroplasts
- ***Campylobacter jejuni* gastroenteritis** gastroenteritis caused by *C. jejuni*; generally mild but sometimes with serious complications
- **candidiasis** fungal infection caused by *Candida* spp., especially *C. albicans*; can affect various regions of the body, e.g., skin (cutaneous candidiasis), oral cavity (oral thrush), or vagina (yeast infection)
- **candle jar** container with a tight-fitting lid in which a burning candle consumes oxygen and releases carbon dioxide, thereby creating an environment suitable for capnophiles
- **capillary** small blood vessel found in the interstitial space of tissue; delivers nutrients and oxygen, and removes waste products
- **capnophile** organism that requires carbon dioxide levels higher than atmospheric concentration

- **capsid** protein coat surrounding the genome of the virus
- **capsomere** individual protein subunits that make up the capsid
- **capsule staining** a negative staining technique that stains around a bacterial capsule while leaving the capsule clear
- **capsule** type of glycocalyx with organized layers of polysaccharides that aid in bacterial adherence to surfaces and in evading destruction by immune cells
- **carbapenem-resistant Enterobacteriaceae (CRE)** group of bacteria that have developed resistance to all β -lactams, including carbapenems, and many other drug classes
- **carbohydrate** the most abundant type of biomolecule, consisting of carbon, hydrogen, and oxygen
- **carbon skeleton** chain of carbon atoms to which one or more functional groups are bound
- **carboxysome** an inclusion composed of an outer shell of thousands of protein subunits. Its interior is filled with ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and carbonic anhydrase, which are both used for carbon metabolism
- **carbuncle** abscess containing a large, deep, purulent skin lesion
- **carcinogen** agent that causes cancer
- **case-control study** a type of observational study in which a group of affected individuals are compared, usually retrospectively, to a similar group of unaffected individuals
- **catabolic activator protein (CAP)/cAMP receptor protein (CRP)** protein that, when bound to cAMP in the presence of low levels of glucose, binds to the promoters of operons that control the processing of alternative sugars
- **catabolism** chemical reactions that break down complex molecules into simpler ones
- **catabolite repression** repression of the transcription of operons encoding enzymes for the use of substrates other than glucose when glucose levels are high
- **catalase** enzyme that breaks down hydrogen peroxide to water and oxygen
- **catalyst** molecule that increases the rate of a chemical reaction but is not used or changed during the chemical reaction and, thus, is reusable

- **catarrhal stage** in pertussis, a disease stage marked by inflammation of the mucous membranes combined with excessive secretions
- **cat-scratch disease** bacterial infection of the lymph nodes caused by *Bartonella henselae*; frequently transmitted via a cat scratch
- **causative agent** the pathogen or substance responsible for causing a particular disease; etiologic agent
- **CCA amino acid binding end** region of a mature tRNA that binds to an amino acid
- **celiac disease** disease largely of the small intestine caused by an immune response to gluten that results in the production of autoantibodies and an inflammatory response
- **cell envelope** the combination of external cellular structures (e.g., plasma membrane, cell wall, outer membrane, glycocalyxes) that collectively contain the cytoplasm and internal structures of a cell
- **cell membrane** lipid bilayer with embedded proteins and carbohydrates that defines the boundary of the cell (also called the cytoplasmic membrane or plasma membrane)
- **cell morphology** cell shape, structure, and arrangement, as viewed microscopically
- **cell theory** the theory that all organisms are composed of cells and that the cell is the fundamental unit of life
- **cell wall** a structure in the cell envelope of some cells that helps the cell maintain its shape and withstand changes in osmotic pressure
- **cellular immunity** adaptive immunity involving T cells and the destruction of pathogens and infected cells
- **cellulitis** a subcutaneous skin infection that develops in the dermis or hypodermis, resulting in a red, painful inflammation
- **cellulose** a structural polysaccharide composed of glucose monomers linked together in a linear chain by glycosidic bonds
- **Centers for Disease Control and Prevention (CDC)** the national public health agency in the United States
- **central dogma** scientific principle explaining the flow of genetic information from DNA to RNA to protein
- **central nervous system (CNS)** portion of the nervous system made up of the brain and spinal cord
- **central tolerance** negative selection of self-reactive T cells in thymus

- **centriole** a component of a centrosome with the structural array of nine parallel microtubules arranged in triplets; involved in eukaryotic cell division
- **centrosome** a microtubule-organizing center for the mitotic spindle found in animal cells; it separates chromosomes during cell division and is composed of a pair of centrioles positioned at right angles to each other
- **cephalosporins** a group of cell wall synthesis inhibitors within the class of β -lactams
- **cercarial dermatitis** inflammation of the skin caused by a reaction to cercaria of *Schistosoma* spp., which can penetrate the skin and blood vessels; also called swimmer's itch or clam digger's itch
- **cerebrospinal fluid (CSF)** sterile liquid produced in the brain that fills the subarachnoid space of the brain and spinal column
- **cervix** the part of the uterus that connects to the vagina
- **CFB group** phylum consisting of the gram-negative, rod-shaped nonproteobacteria genera *Cytophaga*, *Fusobacterium*, and *Bacteroides*
- **Chagas disease** potentially fatal protozoan infection caused by *Trypanosoma cruzi* and endemic to Central and South America; transmitted by the triatomine bug (kissing bug)
- **chancroid** an STI caused by *Haemophilus ducreyi* that produces soft chancres on genitals
- **charged tRNA** activated tRNA molecule carrying its cognate amino acid
- **chemical mediators** chemicals or enzymes produced by a variety of cells; provide nonspecific antimicrobial defense mechanisms
- **chemically defined media** media in which all components are chemically defined
- **chemiosmosis** flow of hydrogen ions across the membrane through ATP synthase
- **chemokines** chemotactic cytokines that recruit specific subsets of leukocytes to infections, damaged tissue, and sites of inflammation
- **chemotaxis** directional movement of a cell in response to a chemical attractant
- **chemotroph** organism that gets its energy from the transfer of electrons originating from chemical compounds

- **chickenpox** common childhood disease caused by the varicella-zoster virus and marked by the formation of pustular lesions on the trunk
- **chikungunya fever** mosquito-borne viral disease caused by the chikungunya virus and characterized by high fever, joint pain, rash, and blisters
- **chirality** property of stereoisomer molecules by which their structures are nonsuperimposable mirror-images
- **chitin** polysaccharide that is an important component of fungal cell walls
- **chlamydia** a common STI caused by *Chlamydia trachomatis*
- **chloramphenicol** protein synthesis inhibitor with broad-spectrum activity that binds to the 50S subunit, inhibiting peptide bond formation
- **chlorophyll** a type of photosynthetic pigment found in some prokaryotic and eukaryotic cells
- **chloroplast** organelle found in plant and algal cells in which photosynthesis occurs
- **cholera** gastrointestinal illness caused by *Vibrio cholera* characterized by severe diarrhea
- **chromatin** combination of DNA with DNA binding proteins
- **chromogenic substrate** colorless substrate (chromogen) that is converted into a colored end product by the enzyme
- **chromophores** pigments that absorb and reflect particular wavelengths of light (giving them a color)
- **chromosome** discrete DNA structure within a cell that controls cellular activities
- **chronic disease** any disease that progresses and persists over a long time
- **chronic granulomatous disease** primary immunodeficiency caused by an impaired ability of phagocytic cells to kill ingested bacteria in the phagolysosome
- **chronic wasting disease** prion disease of deer and elk in the United States and Canada
- **cilia (singular: cilium)** short filamentous structures found on some eukaryotic cells; each is composed of microtubules in a 9+2 array, and may be used for locomotion, feeding, and/or movement of extracellular particles that come in contact with the cell

- **ciliated epithelial cells** hair-like cells in the respiratory tract that beat, pushing mucus secretions and trapped debris away from the sensitive tissues of the lungs
- **ciliates** protists with cilia (Ciliophora), including *Paramecium* and *Stentor*, classified within the Chromalveolata
- **cisternae** the sacs of the endoplasmic reticulum
- **citric acid cycle** see *Krebs cycle*
- **class switching** genetic rearrangement of constant region gene segments in plasma cells to switch antibody production from IgM to IgG, IgA, or IgE
- **clindamycin** semisynthetic protein synthesis inhibitor of the lincosamide class that binds to the 50S subunit, inhibiting peptide bond formation
- **clone** a genetically identical cell or individual
- ***Clostridium perfringens* gastroenteritis** relatively mild gastrointestinal illness caused by *C. perfringens*
- **clusters of differentiation (CD)** cell-surface glycoproteins that serve to identify and distinguish white blood cells
- **coagulase** enzyme that causes the activation of fibrinogen to form fibrin, promoting clotting of the blood
- **coarse focusing knob** a knob on a microscope that produces relatively large movements to adjust focus
- **coccidioidomycosis** disease caused by the highly infectious fungal pathogen *Coccidioides immitis* and related species
- **codon** three-nucleotide sequence within mRNA that specifies a particular amino acid to be incorporated into the polypeptide being synthesized
- **coenocyte** multinucleated eukaryotic cell that forms as a result of multiple rounds of nuclear division without the accompanying division of the plasma membrane
- **coenocytic hyphae** nonseptate hyphae that are multinucleate and lack cell walls or membranes between cells; characteristic of some fungi
- **coenzyme** organic molecule required for proper enzyme function that is not consumed and is reusable
- **cofactor** inorganic ion that helps stabilize enzyme conformation and function

- **cognate amino acid** amino acid added to a specific tRNA molecule that correctly corresponds to the tRNA's anticodon and, hence, the mRNA's codon, reflecting the genetic code
- **cohort method** a method used in observational studies in which a group of individuals is followed over time and factors potentially important in the development of disease are evaluated
- **colistin** membrane-active polymyxin that was historically used for bowel decontamination but now used for systemic infections with drug-resistant pathogens
- **colitis** inflammation of the large intestine
- **collagenase** enzyme that digests collagen, the dominant protein in connective tissue
- **colony-forming unit (CFU)** a counting quantity represented by a colony formed on solid medium from a single cell or a few cells
- **commensalism** type of symbiosis in which one population benefits and the other is not affected
- **commercial sterilization** type of sterilization protocol used in food production; uses conditions that are less harsh (lower temperatures) to preserve food quality but still effectively destroy vegetative cells and endospores of common foodborne pathogens such as *Clostridium botulinum*
- **common cold** most common cause of rhinitis in humans; associated with a variety of adenoviruses, coronaviruses, and rhinoviruses
- **common source spread** a mode of disease transmission in which every infection originates from the same source
- **communicable** able to be transmitted directly or indirectly from one person to another
- **community** group of interacting populations of organisms
- **competitive inhibitor** molecule that binds to an enzyme's active site, preventing substrate binding
- **competitive interactions** interactions between populations in which one of them competes with another for resources
- **complement activation** cascading activation of the complement proteins in the blood, resulting in opsonization, inflammation, and lysis of pathogens
- **complement fixation test** test for antibodies against a specific pathogen using complement-mediated hemolysis

- **complement system** series of proteins that can become activated in the presence of invading microbes, resulting in opsonization, inflammation, and lysis of pathogens
- **complementary base pairs** base pairing due to hydrogen bonding that occurs between a specific purine and a specific pyrimidine; A bonds with T (in DNA), and C bonds with G
- **complementary DNA (cDNA)** a DNA molecule complementary to mRNA that is made through the activity of reverse transcriptase
- **complex media** media that contain extracts of animals and plants that are not chemically defined
- **complex virus** virus shape that often includes intricate characteristics not seen in the other categories of capsid
- **compound microscope** a microscope that uses multiple lenses to focus light from the specimen
- **condenser lens** a lens on a microscope that focuses light from the light source onto the specimen
- **conditional mutation** mutant form of a gene whose mutant phenotype is expressed only under certain environmental conditions
- **confocal microscope** a scanning laser microscope that uses fluorescent dyes and excitation lasers to create three-dimensional images
- **conidia** asexual fungal spores not enclosed in a sac; produced in a chain at the end of specialized hyphae called conidiophores
- **conjugate vaccine** a vaccine consisting of a polysaccharide antigen conjugated to a protein to enhance immune response to the polysaccharide; conjugate vaccines are important for young children who do not respond well to polysaccharide antigens
- **conjugated protein** protein carrying a nonpolypeptidic portion
- **conjugation** mechanism of horizontal gene transfer in bacteria in which DNA is directly transferred from one bacterial cell to another by a conjugation pilus
- **conjugation pilus (sex pilus)** hollow tube composed of protein encoded by the conjugation plasmid that brings two bacterial cells into contact with each other for the process of conjugation
- **conjunctiva** the mucous membranes covering the eyeball and inner eyelid
- **conjunctivitis** inflammation of the conjunctiva, the mucous membrane covering the eye and inside of the eyelid

- **constitutively expressed** describes genes that are transcribed and translated continuously to provide the cell with constant intermediate levels of the protein products
- **contact dermatitis** inflammation of the skin resulting from a type IV hypersensitivity to an allergen or irritant
- **contact** see *exposure*
- **contact transmission** movement of a pathogen between hosts due to contact between the two; may be direct or indirect
- **contagious** easily spread from person to person
- **continuous cell line** derived from transformed cells or tumors, these cells are often able to be subcultured many times, or, in the case of immortal cell lines, grown indefinitely
- **continuous common source spread** a mode of disease transmission in which every infection originates from the same source and that source produces infections for longer than one incubation period
- **contractile vacuoles** organelles found in some cells, especially in some protists, that take up water and then move the water out of the cell for osmoregulatory purposes (i.e., to maintain an appropriate salt and water balance)
- **contrast** visible differences between parts of a microscopic specimen
- **convalescence stage** the final stage of a whooping cough infection, marked by a chronic cough
- **Coombs' reagent** antiserum containing antihuman immunoglobulins used to facilitate hemagglutination by cross-linking the human antibodies attached to red blood cells
- **cooperative interactions** interactions between populations in which both benefit
- **cortex** tightly packed layer of fungal filaments at the outer surface of a lichen; foliose lichens have a second cortex layer beneath the medulla
- **counterstain** a secondary stain that adds contrasting color to cells from which the primary stain has been washed out by a decolorizing agent
- **crenation** shriveling of a cell
- **Creutzfeldt-Jakob disease** form of transmissible spongiform encephalopathy found in humans; typically a fatal disease
- **crisis phase** point at which a fever breaks, reaching a peak before the hypothalamus resets back to normal body temperature

- **critical item** object that must be sterile because it will be used inside the body, often penetrating sterile tissues or the bloodstream
- **cross-match** in the major cross-match, donor red blood cells are checked for agglutination using recipient serum; in the minor cross-match, donor serum is checked for agglutinating antibodies against recipient red blood cells
- **cross-presentation** a mechanism by which dendritic cells process antigens for MHC I presentation to CD8 T cells through phagocytosis of the pathogen (which would normally lead to MHC II presentation)
- **cross-resistance** when a single resistance mechanism confers resistance to multiple antimicrobial drugs
- **cross-sectional study** a type of observational study in which measurements are made on cases, both affected and unaffected, at one point in time and the measurements analyzed to uncover associations with the disease state
- **crustose lichens** lichens that are tightly attached to the substrate, giving them a crusty appearance
- **cryptococcosis** fungal pneumonia caused by the encapsulated yeast *Cryptococcus neoformans* commonly found in bird droppings
- **cryptosporidiosis** intestinal infection caused by *Cryptosporidium parvum* or *C. hominis*
- **culture density** the number of cells per volume of broth
- **culture medium** combination of compounds in solution that supports growth
- **cutaneous mycosis** any fungal infection that affects the surface of the skin, hair, or nails
- **cyanobacteria** phototrophic, chlorophyll-containing bacteria that produce large amounts of gaseous oxygen
- **cyclic AMP (cAMP)** intracellular signaling molecule made through the action of adenylyl cyclase from ATP when glucose levels are low, with the ability to bind to a catabolite activator protein to allow it to bind to regulatory regions and activate the transcription of operons encoding enzymes for metabolism of alternative substrates
- **cyclic photophosphorylation** pathway used in photosynthetic organisms when the cell's need for ATP outweighs that for NADPH, thus bypassing NADPH production
- **cyclosporiasis** intestinal infection caused by *Cyclospora cayetanensis*

- **cystic echinococcosis** hydatid disease, an infection caused by the tapeworm *Echinococcus granulosus* that can cause cyst formation
- **cysticerci** larval form of a tapeworm
- **cystitis** inflammation of the bladder
- **cysts** microbial cells surrounded by a protective outer covering; some microbial cysts are formed to help the microbe survive harsh conditions, whereas others are a normal part of the life cycle
- **cytochrome oxidase** final ETS complex used in aerobic respiration that transfers energy-depleted electrons to oxygen to form H₂O
- **cytokine storm** an excessive release of cytokines, typically triggered by a superantigen, that results in unregulated activation of T cells
- **cytokines** protein molecules that act as a chemical signals; produced by cells in response to a stimulation event
- **cytomegalovirus (CMV) infection** human herpesvirus 5 infection that is typically asymptomatic but can become serious in immunocompromised patients, transplant recipients, and developing fetuses
- **cytopathic effect** cell abnormality resulting from a viral infection
- **cytoplasm** the gel-like material composed of water and dissolved or suspended chemicals contained within the plasma membrane of a cell
- **cytoplasmic membrane** see *cell membrane*
- **cytoproct** a protozoan cell structure that is specialized for excretion
- **cytosine** pyrimidine nitrogenous base found in nucleotides
- **cytoskeleton** a network of filaments or tubules in the eukaryotic cell that provides shape and structural support for cells; aids movement of materials throughout the cell
- **cytostome** a protozoan cell structure that is specialized for phagocytosis (i.e., to take in food)
- **cytotoxic T cells** effector cells of cellular immunity that target and eliminate cells infected with intracellular pathogens through induction of apoptosis
- **cytotoxicity** harmful effects to host cell

D

- **dacryocystitis** inflammation of the lacrimal sac often associated with a plugged nasolacrimal duct
- **daptomycin** cyclic lipopeptide that disrupts the bacterial cell membrane
- **darkfield microscope** a compound light microscope that produces a bright image on a dark background; typically a modified brightfield microscope
- **death phase (decline phase)** phase of the growth curve at which the number of dying cells exceeds the number of new cells formed
- **decimal reduction time (DRT) or D-value** amount of time it takes for a specific protocol to produce a one order of magnitude decrease in the number of organisms; that is, death of 90% of the population
- **decolorizing agent** a substance that removes a stain, usually from some parts of the specimen
- **deeply branching bacteria** bacteria that occupy the lowest branches of the phylogenetic tree of life
- **definitive host** the preferred host organism for a parasite, in which the parasite reaches maturity and may reproduce sexually
- **degeneracy** redundancy in the genetic code because a given amino acid is encoded by more than one nucleotide triplet codon
- **degerming** protocol that significantly reduces microbial numbers by using mild chemicals (e.g., soap) and gentle scrubbing of a small area of skin or tissue to avoid the transmission of pathogenic microbes
- **degranulation** release of the contents of mast cell granules in response to the cross-linking of IgE molecules on the cell surface with allergen molecules
- **dehydration synthesis** chemical reaction in which monomer molecules bind end to end in a process that results in the formation of water molecules as a byproduct
- **deletion** type of mutation involving the removal of one or more bases from a DNA sequence
- **Deltaproteobacteria** class of Proteobacteria that includes sulfate-reducing bacteria
- **denatured protein** protein that has lost its secondary and tertiary structures (and quaternary structure, if applicable) without the loss of its primary structure
- **dendrites** branched extensions of the soma of a neuron that interact with other cells

- **dengue fever** mosquito-borne viral hemorrhagic disease; also known as breakbone fever
- **dental calculus** calcified heavy plaque on teeth, also called tartar
- **dental caries** cavities formed in the teeth as a result of tooth decay caused by microbial activity
- **deoxyribonucleic acid (DNA)** double-stranded nucleic acid composed of deoxyribonucleotides that serves as the genetic material of the cell
- **deoxyribonucleotides** DNA nucleotides containing deoxyribose as the pentose sugar component
- **dermatophyte** any fungus of the genera *Microsporum*, *Epidermophyton*, or *Trichophyton*, which feed on keratin (a protein found in skin, hair, and nails) and can cause cutaneous infections
- **dermis** the second layer of human skin, found between the epidermis and the hypodermis
- **descriptive epidemiology** a method of studying a disease outbreak using case histories, contact interviews, medical information, and other sources of information
- **desensitization** injections of antigen that lead to production of antigen-specific IgG molecules, effectively outcompeting IgE molecules on the surface of sensitized mast cells for antigen
- **desiccation** method of microbial control involving the removal of water from cells through drying or dehydration
- **desquamation** peeling and shedding of outermost skin
- **diapedesis** process by which leukocytes pass through capillary walls to reach infected tissue; also called extravasation
- **diaphragm** a component of a microscope; typically consists of a disk under the stage with holes of various sizes; can be adjusted to allow more or less light from the light source to reach the specimen
- **differential interference-contrast microscope** a microscope that uses polarized light to increase contrast
- **differential media** media that contain additives that make it possible to distinguish bacterial colonies based on metabolic activities of the organisms
- **differential staining** staining that uses multiple dyes to differentiate between structures or organisms
- **diffraction** the changing of direction (bending or spreading) that occurs when a light wave interacts with an opening or barrier

- **dikaryotic** having two separate nuclei within one cell
- **dimorphic fungus** a fungus that can take the form of a yeast or a mold, depending on environmental conditions
- **refers to sexually reproducing organisms in which individuals have either male or female reproductive organs (not both)**
- **diphtheria** serious infection of the larynx, caused by the toxigenic bacterium *Corynebacterium diphtheriae*
- **diploid** having two copies of each chromosome
- **direct agglutination assay** assay that can be used to detect the agglutination of bacteria by the action of antibodies in patient serum
- **direct antihuman globulin test (DAT)** another name for a direct Coombs' test
- **direct contact transmission** movement of a pathogen between hosts by physical contact or transfer in droplets at a distance less than one meter
- **direct Coombs' test** assay that looks for antibodies *in vivo* against red blood cells caused by various types of infections, drug reactions, and autoimmune disorders
- **direct ELISA** enzyme-linked immunoabsorbent assay in which the antigens are immobilized in the well of a microtiter plate; only a single antibody is used in the test
- **direct fluorescent antibody (DFA) test** FA technique in which the labeled antibody binds to the target antigen
- **direct hemagglutination assay** test that determines the titer of certain bacteria and viruses that causes clumping of red blood cells
- **direct microscopic cell count** counting of cells using a calibrated slide under a light microscope
- **direct repair (light repair or photoreactivation)** light-dependent mechanism for repairing pyrimidine dimers involving the enzyme photolyase
- **disaccharide** one of two monosaccharides linked together by a glycosidic bond
- **disease** any condition in which the normal structure or function of the body is damaged or impaired
- **disinfectant** antimicrobial chemical applied to a fomite during disinfection that may be toxic to tissues
- **disinfection** protocol that removes potential pathogens from a fomite

- **disk-diffusion method** a technique for measuring of the effectiveness of one or more antimicrobial agents against a known bacterium; involves measuring the zone(s) of inhibition around the chemical agent(s) in a culture of the bacterium
- **dispersion** the separation of light of different frequencies due to different degrees of refraction
- **disulfide bridge** covalent bond between the sulfur atoms of two sulfhydryl side chains
- **DNA gyrase (topoisomerase II)** bacterial topoisomerase that relaxes the supercoiled chromosome to make DNA more accessible for the initiation of replication
- **DNA ligase** enzyme that catalyzes the formation of a covalent phosphodiester linkage between the 3'-OH end of one DNA fragment and the 5' phosphate end of another DNA fragment
- **DNA packaging** process in which histones or other DNA binding proteins perform various levels of DNA wrapping and attachment to scaffolding proteins to allow the DNA to fit inside a cell
- **DNA polymerase** class of enzymes that adds nucleotides to the free 3'-OH group of a growing DNA chain that are complementary to the template strand
- **DNA primers** short, synthetic, single-stranded DNA fragments of known sequence that bind to specific target sequences within a sample due to complementarity between the target DNA sequence and the primer; commonly used in PCR but may be used in other hybridization techniques
- **DNA probe** a single-stranded DNA fragment that is complementary to part of the gene (DNA or RNA) of interest
- **DNAse** pathogen-produced nuclease that degrades extracellular DNA
- **dosage** amount of medication given during a certain time interval
- **double immunodiffusion** see Ouchterlony assay
- **doubling time** the time it takes for the population to double; also referred to as generation time
- **droplet transmission** direct contact transmission of a pathogen transferred in sneezed or coughed droplets of mucus that land on the new host within a radius of one meter
- **drug resistance** ability of a microbe to persist and grow in the presence of an antimicrobial drug

- **dry-heat sterilization** protocol that involves the direct application of high heat
- **dura mater** tough, outermost membrane that surrounds the brain
- **dynein** motor proteins that interact with microtubules in eukaryotic flagella and cilia
- **dysentery** intestinal inflammation that causes diarrhea with blood and mucus
- **dysuria** urination accompanied by burning, discomfort, or pain

E

- **E (exit) site** functional site of an intact ribosome that releases dissociated uncharged tRNAs so that they can be recharged with free amino acids
- **East African trypanosomiasis** acute form of African trypanosomiasis caused by *Trypanosoma brucei rhodesiense*
- **eastern equine encephalitis** serious, but rare, mosquito-borne viral infection of the brain that is found primarily on the Atlantic and Gulf coast states of the United States
- **Ebola virus disease (EVD)** potentially fatal viral hemorrhagic fever found primarily in western Africa and transmitted through contact with body fluids
- **eclipse phase** period after viral infection during which the infective virus is not detected, either intracellularly or extracellularly, and biosynthesis is occurring
- **ectoplasm** outer, more gelatinous layer of cytoplasm under a protist cell membrane
- **edema** swelling due to accumulation of fluid and protein in tissue as a result of increased permeability of capillary walls during an inflammatory response; chronic edema can also result from blockage of lymphatic vessels, as in the case of elephantiasis
- **effector cells** activated cells of cellular immunity that are involved in the immediate immune response, primarily to defend the body against pathogens
- **electron carrier** cellular molecule that accepts high-energy electrons from reduced molecules like foods and later serves as an electron

donor in subsequent redox reactions

- **electron microscope** a type of microscope that uses short-wavelength electron beams rather than light to increase magnification and resolution
- **electron transport system (ETS)** series of membrane-associated protein complexes and associated mobile accessory electron carriers important in the generation of the proton motive force required for ATP production by chemiosmosis; the last component involved in the cellular respiration of glucose
- **electroporation** a genetic engineering technique in which cells are exposed to a short electric pulse, inducing them to take up DNA molecules from their environment
- **elementary bodies** metabolically and reproductively inactive, endospore-like form of intracellular bacteria that spreads infection outside of cells
- **elongation in DNA replication** stage of DNA replication during which DNA polymerase adds nucleotides, complementary to the parental strand, to the 3' end of a growing DNA strand
- **elongation in transcription** stage of transcription during which RNA polymerase extends the RNA molecule by adding RNA nucleotides, complementary to the template DNA strand
- **elongation of translation** stage of translation during which amino acids are added one by one to the C-terminus of the growing polypeptide
- **Embden-Meyerhof-Parnas (EMP) pathway** type of glycolysis found in animals and the most common in microbes
- **emerging infectious disease** a disease that is new to the human population or has increased in prevalence over the previous 20 years
- **enantiomers** stereoisomers that are mirror images of each other and nonsuperimposable
- **encephalitis** inflammation of the tissues of the brain
- **encystment** the process of forming a cyst
- **endemic disease** an illness that is constantly present (often at low levels) in a population
- **endergonic reaction** chemical reaction that requires energy beyond activation energy to occur

- **endocarditis** inflammation of the endocardium, especially the heart valves
- **endocrine function** refers to a cytokine signal released from a cell and carried by the bloodstream to a distant recipient cell
- **endocytosis** the uptake of molecules through plasma membrane invagination and vacuole/vesicle formation
- **endomembrane system** a series of organelles (endoplasmic reticulum, Golgi apparatuses, lysosomes, and transport vesicles) arranged as membranous tubules, sacs, and disks that synthesize many cell components
- **endoplasm** inner, more fluid layer of cytoplasm under a protist cell membrane (inside of the ectoplasm)
- **endoplasmic reticulum** part of the endomembrane system that is an interconnected array of tubules and flattened sacs with a single lipid bilayer that may be either rough or smooth; important in synthesizing proteins and lipids
- **endospore** a cellular structure formed by some bacteria in response to adverse conditions; preserves DNA of the cell in a dormant state until conditions are favorable again
- **endospore staining** a differential staining technique that uses two stains to make bacterial endospores appear distinct from the rest of the cell
- **endosymbiotic theory** the theory that mitochondria and chloroplasts arose as a result of prokaryotic cells establishing a symbiotic relationship within a eukaryotic host
- **endothelia** layer of epithelial cells lining blood vessels, lymphatics, the blood-brain barrier, and some other tissues
- **endotoxin** lipid A component of lipopolysaccharides in the outer membrane of gram-negative bacteria
- **enriched media** media that contain additional essential nutrients to support growth
- **enrichment culture** media providing growth conditions that favor the expansion of an organism present in low numbers
- **enteric** bacteria of the family Enterobacteriaceae, which live in the human intestinal tract
- **enteritis** inflammation of the lining of the intestine

- **enterobiasis** intestinal infection caused by the pinworm *Enterobius vermicularis*
- **enterohemorrhagic *E. coli* (EHEC)** *E. coli* bacteria that cause severe gastrointestinal illness with potential serious complications such as hemolytic uremic syndrome
- **enteroinvasive *E. coli* (EIEC)** *E. coli* bacteria that cause relatively mild gastrointestinal illness
- **enteropathogenic *E. coli* (EPEC)** *E. coli* bacteria that cause serious gastrointestinal illness
- **enterotoxigenic *E. coli* (ETEC)** *E. coli* bacteria that cause a relatively mild illness commonly called traveler's diarrhea
- **enterotoxin** toxin that affects the intestines
- **Entner-Doudoroff (ED) pathway** alternative glycolytic pathway used by some bacteria
- **enveloped virus** a virus formed with a nucleic-acid packed capsid surrounded by a lipid layer
- **enzyme** catalyst for biochemical reactions inside cells
- **enzyme immunoassay (EIA)** type of assay wherein an enzyme is coupled to an antibody; addition of a chromogenic substrate for the antibody allows quantification or identification of the antigen bound by the antibody
- **enzyme-linked immunosorbent assay (ELISA)** specialized form of EIA in which either the primary antibody or the antigen is first attached to a solid surface such as the well of a microtiter plate
- **eosinophils** leukocytes with granules containing histamine and major basic protein; facilitate allergic responses and protection against parasitic protozoa and helminths
- **epidemic disease** an illness with a higher-than-expected incidence in a given period within a given population
- **epidemic typhus** severe and sometimes fatal infection caused by *Rickettsia prowazekii* and transmitted by body lice
- **epidemiology** the study of where and when infectious diseases occur in a population and how they are transmitted and maintained in nature
- **epidermis** the outermost layer of human skin
- **epididymis** coiled tube that collects sperm from the testes and passes it on to the vas deferens

- **epididymitis** inflammation of the epididymis caused by a bacterial infection
- **epigenetic regulation** chemical modification of DNA or associated histones to influence transcription
- **epiglottis** flap of cartilage that covers the larynx during swallowing; diverts food to the esophagus and prevents it from entering the respiratory tract
- **epiglottitis** inflammation of the epiglottis
- **epiphyte** a plant that grows on another plant
- **epitope** smaller exposed region on an antigen that is recognized by B-cell and T-cell receptors and antibodies
- **Epsilonproteobacteria** class of Proteobacteria that are microaerophilic
- **equivalence zone** region where the antibody–antigen ratio produces the greatest amount of precipitin in a precipitin reaction
- **erysipelas** a skin infection, typically caused by *Streptococcus pyogenes*, that presents as a red, large, intensely inflamed patch of skin involving the dermis, usually with clear borders, typically on the legs or face
- **erythema nodosum** a condition that causes inflammation in the subcutaneous fat cells of the hypodermis resulting in red nodules
- **erythema** redness at the site of inflammation, usually due to dilation of blood vessels in the area to help bring in white blood cells
- **erythrocyte** red blood cell
- **erythrogenic toxin** exotoxin produced by some strains of *Streptococcus pyogenes*; activity of the toxin can produce the characteristic rash of scarlet fever
- **erythromycin** protein synthesis inhibitor of the macrolide class that is often used as an alternative to penicillin
- **eschar** a localized mass of dead skin tissue
- **Etest** simple, rapid method for determining MIC, involving commercially available plastic strips that contain a gradient of an antimicrobial and are placed on an agar plate inoculated with a bacterial lawn
- **etiological agent** the pathogen or substance responsible for causing a particular disease; causative agent
- **etiology** the science of the causes of disease

- **Eukarya** the domain of life that includes all unicellular and multicellular organisms with cells that contain membrane-bound nuclei and organelles
- **eukaryote** an organism made up of one or more cells that contain a membrane-bound nucleus and organelles
- **eukaryotic cell** has a nucleus surrounded by a complex nuclear membrane that contains multiple, rod-shaped chromosomes
- **eustachian tube** small passage between the nasopharynx and the middle ear that allows pressure to equalize across the tympanic membrane
- **eutrophs** microorganisms that require a copious amount of organic nutrients; also called copiotrophs
- **excystment** the process of emerging from a cyst
- **exergonic reaction** chemical reaction that does not require energy beyond activation energy to proceed; releases energy when the reaction occurs
- **exocytosis** the release of the contents of transport vesicles to the cell's exterior by fusion of the transport vesicle's membrane with the plasma membrane
- **exoenzyme** secreted enzyme that enhances the ability of microorganisms to invade host cells
- **exon** protein-coding sequence of a eukaryotic gene that is transcribed into RNA and spliced together to code for a polypeptide
- **exonuclease** enzymatic activity that removes RNA primers in DNA introduced by primase
- **exotoxin** biologically active product that causes adverse changes in the host cells
- **experimental epidemiology** the use of laboratory and clinical studies to directly study disease in a population
- **experimental study** a type of scientific study that involves manipulation of the study subjects by the researcher through application of specific treatments hypothesized to affect the outcome while maintaining rigorously controlled conditions
- **exposure** contact between potential pathogen and host; also called contamination or contact
- **extended-spectrum β -lactamases (ESBLs)** β -lactamases carried by some gram-negative bacteria that provide resistance to all penicillins,

cephalosporins, monobactams, and β -lactamase-inhibitor combinations, but not carbapenems

- **extensively drug resistant *Mycobacterium tuberculosis* (XDR-TB)** strains of *M. tuberculosis* that are resistant to rifampin and isoniazid, and also are resistant to any fluoroquinolone and at least one of three other drugs (amikacin, kanamycin, or capreomycin)
- **extracellular matrix** material composed of proteoglycans and fibrous proteins secreted by some eukaryotic cells that lack cell walls; helps multicellular structures withstand physical stresses and coordinates signaling from the external surface of the cell to the interior of the cell
- **extracellular polymeric substances (EPS)** hydrated gel secreted by bacteria in a biofilm containing polysaccharides, proteins, nucleic acids, and some lipids
- **extrachromosomal DNA** additional molecules of DNA distinct from the chromosomes that are also part of the cell's genome
- **extravasation** process by which leukocytes pass through capillary walls to reach infected tissue; also called diapedesis

F

- **F⁻ (recipient) cell** *E. coli* cell lacking the F plasmid and thus incapable of forming a conjugation pilus but capable of receiving the F plasmid during conjugation
- **F pilus (F pili)** specialized type of pilus that aids in DNA transfer between cells; conjugation pilus of *E. coli*
- **F plasmid (fertility factor)** bacterial plasmid in *E. coli* containing genes encoding the ability to conjugate, including genes encoding the formation of the conjugation pilus
- **F' plasmid** integrated F plasmid imprecisely excised from the chromosome; carries with it some chromosomal DNA adjacent to the integration site
- **F⁺ (donor) cell** *E. coli* cell containing the F plasmid, capable of forming a conjugation pilus
- **Fab region** arm of an antibody molecule that includes an antigen-binding site

- **facultative anaerobe** organism that grows better in the presence of oxygen but can proliferate in its absence
- **false negative** negative result to a test for an infection or condition (e.g., presence of antigen, antibody, or nucleic acid) when the infection or condition is actually present
- **false positive** positive result to a test for an infection or condition (e.g., presence of antigen, antibody, or nucleic acid) when the infection or condition is actually absent
- **fastidious organism** organism that has extensive growth requirements
- **fatty acid** lipid that contains long-chain hydrocarbons terminated with a carboxylic acid functional group
- **fatty acid methyl ester (FAME) analysis** technique in which the microbe's fatty acids are extracted, converted to volatile methyl esters, and analyzed by gas chromatography, yielding chromatograms that may be compared to reference data for identification purposes
- **Fc region** region on the trunk of an antibody molecule involved in complement activation and opsonization
- **feedback inhibition** mechanism of regulating metabolic pathway whereby the product of a metabolic pathway noncompetitively binds to an enzyme early on in the pathway, temporarily preventing the synthesis of the product
- **fermentation** process that uses an organic molecule as a final electron acceptor to regenerate NAD⁺ from NADH such that glycolysis can continue
- **fever** system-wide sign of inflammation that raises the body temperature and stimulates the immune response
- **fifth disease** a highly contagious illness, more commonly affecting children, marked by a distinctive “slapped-cheek” rash and caused by parvovirus B19
- **fimbriae** filamentous appendages found by the hundreds on some bacterial cells; they aid adherence to host cells
- **fine focusing knob** a knob on a microscope that produces relatively small movements to adjust focus
- **fixation** the process by which cells are killed and attached to a slide
- **flagella** long, rigid, spiral structures used by prokaryotic cells for motility in aqueous environments; composed of a filament made of

flagellin, a hook, and motor (basal body) that are attached to the cell envelope

- **flagella staining** a staining protocol that uses a mordant to coat the flagella with stain until they are thick enough to be seen
- **flagellum (eukaryotic) (plural: flagella)** long, whip-like, filamentous external structure found on some eukaryotic cells; composed of microtubules in a 9+2 arrangement; used for locomotion
- **flavin adenine dinucleotide (FAD/FADH₂)** oxidized/reduced forms of an electron carrier in cells
- **flocculant** visible aggregation that forms between a substance in suspension (e.g., lipid in water) and antibodies against the substance
- **flow cytometry** technique analyzing cells for fluorescence intensity; specific subsets of cells are usually labeled in some way prior to the analysis
- **fluconazole** antifungal drug of the imidazole class that is administered orally or intravenously for the treatment of several types of systemic yeast infections
- **fluid mosaic model** refers to the ability of membrane components to move fluidly within the plane of the membrane, as well as the mosaic-like composition of the components
- **flukes** any of the parasitic nonsegmented flatworms (trematodes) that have an oral sucker and sometimes a second ventral sucker; they attach to the inner walls of intestines, lungs, large blood vessels, or the liver in human hosts
- **fluorescence microscope** a microscope that uses natural fluorochromes or fluorescent stains to increase contrast
- **fluorescence-activated cell sorter (FACS)** technique for using a flow cytometer to physically separate cells into two populations based on fluorescence intensity
- **fluorescent antibody (FA) techniques** suite of assays that use a fluorescently labeled antibody to bind to and so make an antigen easy to visualize
- **fluorescent enzyme immunoassay (FEIA)** EIA in which the substrate is a fluorogen that becomes fluorescent following reaction with the enzyme
- **fluorescent** the ability of certain materials to absorb energy and then immediately release that energy in the form of light

- **fluorochromes** chromophores that fluoresce (absorb and then emit light)
- **fluorogen** nonfluorescent molecule that becomes fluorescent on enzyme or laser activation
- **fluorophore** molecule that fluoresces when excited by light
- **fluoroquinolones** class of synthetic antimicrobials that inhibit the activity of DNA gyrase, preventing DNA replication
- **focal infection** infection in which the pathogen causes infection in one location that then spreads to a secondary location
- **focal length** the distance from the lens to the image point when the object is at a definite distance from the lens (this is also the distance to the focal point)
- **focal point** a property of the lens; the image point when light entering the lens is parallel (i.e., the object is an infinite distance from the lens)
- **foliose lichens** lichens that have lobes that may appear to resemble leaves
- **folliculitis** a skin infection characterized by localized inflammation of hair follicles, typically producing an itchy red rash
- **fomite** inanimate item that may harbor microbes and aid in disease transmission
- **foodborne disease** disease that is transmitted through contaminated food
- **fragmentation** newly formed cells split away from the parent filament in actinomycetes and cyanobacteria
- **frameshift mutation** mutation resulting from either an insertion or a deletion in a number of nucleotides that, if not a multiple of three, changes every amino acid after the mutation
- **free ribosome** eukaryotic 80S ribosome found in the cytoplasm; synthesizes water-soluble proteins
- **frequency** the rate of vibration for a light wave or other electromagnetic wave
- **fruticose lichens** lichens that are generally branched with a rounded appearance
- **functional groups** specific groups of atoms that may occur within a molecule, conferring specific chemical properties
- **fungi** (singular: fungus) any of various unicellular or multicellular eukaryotic organisms, typically having cell walls made out of chitin

and lacking photosynthetic pigments, vascular tissues, and organs

- **fungicide** chemical or physical treatment that kills fungi
- **fungistatic** having the ability to inhibit fungal growth, generally by means of chemical or physical treatment
- **furuncle** a small, purulent skin lesion; sometimes called a boil
- **fusion inhibitor** antiviral drug that blocks the fusion of HIV receptors to the coreceptors required for virus entry into the cell, specifically, chemokine receptor type 5

G

- **Gammaproteobacteria** class of Proteobacteria that is very diverse and includes a number of human pathogens
- **gas gangrene** rapidly spreading infection of necrotic tissues caused by the gram-positive anaerobe *Clostridium perfringens* and other *Clostridium* spp.
- **gastritis** inflammation of the lining of the stomach
- **gastroenteritis** inflammation of the lining of the stomach and intestine
- **gene expression** production of proteins from the information contained in DNA through the processes of transcription and translation
- **gene gun** an apparatus that shoots gold or tungsten particles coated with recombinant DNA molecules at high speeds into plant protoplasts
- **gene silencing** a genetic engineering technique in which researchers prevent the expression of a particular gene by using small interfering RNAs (siRNAs) or microRNAs (miRNAs) to interfere with translation
- **gene therapy** a form of treatment for diseases that result from genetic mutations; involves the introduction of nonmutated, functional genes into the genome of the patient, often by way of a viral vector
- **generalized transduction** transfer of a random piece of bacterial chromosome DNA by the phage
- **generation time** see *doubling time*
- **genes** segments of DNA molecules that code for proteins or stable RNA molecules
- **genetic code** correspondence between mRNA nucleotide codons and the translated amino acids

- **genetic engineering** the direct alteration of an organism's genetics to achieve desirable traits
- **genital herpes** an STI caused by the herpes simplex virus
- **genital warts** soft, pink, irregular growths that develop in the external genitalia or anus as a result of human papillomavirus infection
- **genome** entire genetic content of a cell
- **genomic library** a repository of an organism's entire genome maintained as cloned fragments in the genomes of strains of a host organism
- **genomics** the study and comparison of entire genomes, including the complete set of genes, their nucleotide sequence and organization, and their interactions within a species and with other species
- **genotype** full collection of genes that a cell contains within its genome
- **germ theory of disease** the theory that many diseases are the result of microbial infection
- **germination** process of an endospore returning to the vegetative state
- **Ghon complex** calcified lesion containing *Mycobacterium tuberculosis*; forms in the lungs of patients with tuberculosis
- **giardiasis** intestinal infection caused by *Giardia lamblia*
- **gingivitis** inflammation of the gums that can cause bleeding
- **glial cell** assists in the organization of neurons, provides a scaffold for some aspects of neuron function, and aids in recovery from neural injury
- **glomerulonephritis** a type of kidney infection involving the glomeruli of the nephrons
- **glomerulus** capillary bed in the nephron of the kidney that filters blood to form urine
- **glycocalyx** cell envelope structure (either capsules or slime layer) outside the cell wall in some bacteria; allows bacteria to adhere to surfaces, aids in biofilm formation, and provides protection from predation
- **glycogen** highly branched storage polysaccharide in animal cells and bacteria
- **glycolipid** complex lipid that contains a carbohydrate moiety
- **glycolysis** first step in the breakdown of glucose, the most common example of which is the Embden-Meyerhoff-Parnas pathway,

producing two pyruvates, two NADH molecules, and two (net yield) ATP per starting glucose molecule

- **glycopeptides** class of antibiotics that inhibit cell wall synthesis by binding to peptidoglycan subunits and blocking their insertion into the cell wall backbone, as well as blocking transpeptidation
- **glycoprotein** conjugated protein with a carbohydrate attached
- **glycosidic bond** forms between the hydroxyl groups of two sugar molecules
- **Golgi apparatus** an organelle of the endomembrane system that is composed of a series of flattened membranous disks, called dictyosomes, each having a single lipid bilayer, that are stacked together; important in the processing of lipids and proteins
- **gonorrhea** a common STI of the reproductive system caused by *Neisseria gonorrhoeae*
- **graft-versus-host disease** specific type of transplantation reaction in which a transplanted immune system (e.g., a bone marrow transplant) contains APCs and T cells that are activated and attack the recipient's tissue
- **Gram stain procedure** a differential staining technique that distinguishes bacteria based upon their cell wall structure
- **granulocytes** leukocytes found in the peripheral blood that are characterized by numerous granules in the cytoplasm; granulocytes include neutrophils, eosinophils, and basophils
- **granuloma** walled-off area of chronically inflamed tissue containing microbial pathogens, macrophages, and cellular materials unable to be eliminated
- **granulomatous amoebic encephalitis (GAE)** serious brain infection of immunocompromised individuals caused by *Acanthamoeba* or *Balamuthia mandrillaris*
- **granzymes** proteases released from a natural killer cell that enter the cytoplasm of a target cell, inducing apoptosis
- **Graves disease** hyperthyroidism caused by an autoimmune disease affecting thyroid function
- **green nonsulfur bacteria** similar to green sulfur bacteria but use substrates other than sulfides for oxidation
- **green sulfur bacteria** phototrophic, anaerobic bacteria that use sulfide for oxidation and produce large amounts of green bacteriochlorophyll

- **growth curve** a graph modeling the number of cells in a culture over time
- **guanine** purine nitrogenous base found in nucleotides
- **Guillain-Barré syndrome** an autoimmune disease, often triggered by bacterial and viral infections, characterized by the destruction of myelin sheaths around neurons, resulting in flaccid paralysis
- **gummas** granulomatous lesions that develop in tertiary syphilis

H

- **hair follicle** a structure embedded in the dermis from which hair grows
- **halophile** organism that depends on high concentrations of salt in the environment to grow
- **halotolerant** organism that grows in the presence of high salt concentration but does not require it
- **Hansen's Disease** chronic bacterial infection of peripheral nervous tissues caused by the acid-fast bacterium, *Mycobacterium leprae*; also known as leprosy
- **hantavirus pulmonary syndrome** acute lung infection by a hantavirus following inhalation of aerosols from the urine or feces of infected rodents
- **haploid** having one copy of each chromosome
- **hapten** a molecule that is too small to be antigenic alone but becomes antigenic when conjugated to a larger protein molecule
- **hard chancre** a generally painless ulcer that develops at the site of infection in primary syphilis
- **Hashimoto thyroiditis** hypothyroidism caused by an autoimmune disease affecting thyroid function
- **healthcare-associated infection (HAI)** an infection acquired in a hospital or other health-care facility unrelated to the reason for which the patient was initially admitted; nosocomial infection
- **heavy chains** longest identical peptide chains in antibody molecules (two per antibody monomer), composed of variable and constant region segments
- **helical virus** cylindrical or rod shaped

- **helicase** enzyme that unwinds DNA by breaking the hydrogen bonds between the nitrogenous base pairs, using ATP
- **helminth** a multicellular parasitic worm
- **helper T cells** class of T cells that is the central orchestrator of the cellular and humoral defenses of adaptive immunity and the cellular defenses of innate immunity
- **hemagglutination** visible clumping of red blood cells that can be caused by some viruses, bacteria, and certain diseases in which antibodies are produced that bind to self-red blood cells
- **hematopoiesis** formation, development, and differentiation of blood cells from pluripotent hematopoietic stem cells
- **hematuria** condition in which there is blood in the urine
- **hemolysin** class of exotoxin that targets and lyses red blood cells, as well as other cells
- **hemolytic disease of the newborn (HDN)** type II hypersensitivity reaction that occurs when maternal anti-Rh antibodies cross the placenta and target fetal Rh+ red blood cells for lysis
- **hemolytic transfusion reaction (HTR)** condition resulting after an incompatible blood transfusion; caused by type II hypersensitivity reaction and destruction of red blood cells
- **hemorrhagic fever with renal syndrome** serious hemorrhagic fever caused by hantavirus infection
- **HEPA filter** high-efficiency particulate air filter with an effective pore size that captures bacterial cells, endospores, and viruses as air passes through, removing them from the air
- **hepatitis** inflammation of the liver
- **herd immunity** a reduction in disease prevalence brought about when few individuals in a population are susceptible to an infectious agent
- **herpes keratitis** eye infection caused by herpes simplex virus
- **herpes simplex virus type 2 (HSV-2)** the type of herpesvirus most commonly associated with genital herpes
- **herpetic gingivostomatitis** inflammation of the mouth and gums often caused by the HSV-1 virus
- **heterolactic fermentation** process producing a mixture of lactic acid, ethanol and/or acetic acid, and CO₂ as fermentation products; the microbes that do this use pentose phosphate pathway glycolysis, which is why they generate multiple fermentation products

- **heterotroph** organism that uses fixed organic carbon compounds as its carbon source
- **hexose monophosphate shunt** see *pentose phosphate pathway*
- **Hfr cell** *E. coli* cell in which an F plasmid has integrated into the host cell's chromosome
- **high G+C gram-positive bacteria** bacteria that have more than 50% guanine and cytosine nucleotides in their DNA
- **high-energy phosphate bond** bond between the negatively charged phosphate groups that holds a lot of potential energy
- **histamine** proinflammatory molecule released by basophils and mast cells in response to stimulation by other cytokines and chemical mediators
- **histones** DNA-binding proteins found in eukaryotes and archaea that aid in orderly packaging of chromosomal DNA
- **histoplasmosis** fungal disease caused by the dimorphic fungus *Histoplasma capsulatum*
- **holoenzyme** enzyme with a bound cofactor or coenzyme
- **holozoic** refers to protozoans that consume food particles through phagocytosis
- **homolactic fermentation** process producing only lactic acid as a fermentation product; the microbes that do this use Embden-Meyerhof-Parnas glycolysis
- **hookworm infection** soil-transmitted intestinal infection caused by the nematodes *Necator americanus* and *Ancylostoma duodenale*
- **horizontal direct transmission** movement of a pathogen from one host to another (excluding mother to embryo, fetus, or infant) in a population through physical contact or through droplet transmission
- **horizontal gene transfer** introduction of genetic material from one organism to another organism within the same generation
- **host range** the types of host cells that a particular virus is able to infect
- **HTST** high-temperature short-time pasteurization is a method of pasteurization commonly used for milk in which the milk is exposed to a temperature of 72 °C for 15 seconds
- **human African trypanosomiasis** serious infection caused by *Trypanosoma brucei* and spread by the bite of the tsetse fly

- **human granulocytic anaplasmosis** zoonotic tickborne disease caused by the obligate intracellular pathogen *Anaplasma phagocytophilum*
- **human immunodeficiency virus (HIV)** retrovirus responsible for acquired immune deficiency syndrome (AIDS) in humans
- **human papillomavirus (HPV)** a group of common sexually transmitted viruses that may be associated with genital warts or with cervical cancer
- **humanized monoclonal antibodies** chimeric antibodies with mouse variable regions and human constant regions
- **humoral immunity** adaptive immunity mediated by antibodies produced by B cells
- **hyaluronidase** enzyme produced by pathogens that degrades hyaluronic acid between adjacent cells in connective tissue
- **hybridization** the joining of two complementary single-stranded DNA molecules
- **hybridoma** clones of cell produced by fusing a normal B cell with a myeloma cell that is capable of producing monoclonal antibodies indefinitely
- **hydatid disease** cystic echinococcosis, an infection caused by the tapeworm *Echinococcus granulosus*
- **hydrophilic** “water loving”; refers to a polar molecule or portion of a molecule capable of strong attraction to water molecules
- **hydrophobic** “water fearing”; refers to a nonpolar molecule or portion of a molecule not capable of strong attraction to water molecules
- **hypersensitivity pneumonitis (HP)** type III and IV hypersensitivities in the lungs that are caused by environmental or occupational exposure to allergens such as mold and dust
- **hypersensitivity** potentially damaging immune response against an antigen
- **hyperthermophile** a microorganism that has an optimum growth temperature close to the temperature of boiling water
- **hypertonic medium** an environment in which the solute concentration outside a cell exceeds that inside the cell, causing water molecules to move out of the cell, resulting in crenation (shriveling) or plasmolysis.
- **hyphae** tubular, filamentous structures that make up most fungi
- **hypodermis** the layer of tissue under the dermis, consisting primarily of fibrous and adipose connective tissue

- **hypotonic medium** an environment in which the solute concentration inside a cell exceeds that outside the cell, causing water molecules to move into the cell, possibly leading to swelling and possibly lysis

I

- **iatrogenic disease** disease caused by or acquired during a medical procedure
- **icosahedral** three-dimensional, 20-sided structure with 12 vertices
- **IgA** antibody dimer primarily found in breast milk, mucus, saliva, and tears
- **IgD** membrane-body antibody monomer functioning as receptor on the surface of B cells
- **IgE** antibody monomer involved in defense against parasites and allergic reactions
- **IgG** antibody monomer most abundant in serum; able to cross placenta; most versatile class of antibody in terms of function
- **IgM** antibody that is a monomer when functioning as a receptor on surface of B cells but a pentamer when secreted in response to specific pathogens; first antibody to respond during primary and secondary responses
- **illuminator** the light source on a microscope
- **image point (focus)** a property of the lens and the distance of the object to the lens; the point at which an image is in focus (the image point is often called the focus)
- **imidazoles** class of antifungal drugs that inhibit ergosterol biosynthesis
- **immune complex** large group of antigens bound by antibodies; large enough to settle out of fluid suspension
- **immunochromatographic assay** assay in which fluids are pulled through test strips by capillary action and antigen captured by mobile antibody-colored bead conjugates; a second, fixed antibody localizes the colored bead, allowing visualization
- **immunocytochemistry (ICC)** staining technique in which cells are fixed and holes dissolved in the membrane to allow passage of labeled antibodies to bind specific intracellular targets

- **immunoelectrophoresis (IEP)** assay following protein electrophoresis (PAGE) of serum, in which antisera against specific serum proteins are added to troughs cut parallel to the electrophoresis track, causing the formation of precipitin arcs
- **immunofiltration** technique in which antibody or antigen can be concentrated by passing fluids through porous membranes, and target molecules are captured as they pass
- **immunofluorescence** a technique that uses a fluorescence microscope and antibody-specific fluorochromes to determine the presence of specific pathogens in a specimen
- **immunoglobulin** antibody
- **immunohistochemistry (IHC)** staining technique in which labeled antibodies are bound to specific cells in a tissue section
- **immunology** the study of the immune system
- **immunostain** use of EIA technology to deliver stain to particular cells in a tissue (immunohistochemistry) or specific targets within a cell (immunocytochemistry)
- **impetigo** a skin infection that may result in vesicles, blisters, or bullae especially around the mouth, commonly caused by *Staphylococcus aureus*, *S. pyogenes*, or a combination of both *S. aureus* and *S. pyogenes*
- **in vitro** outside the organism in a test tube or artificial environment
- **in vivo** inside the organism
- **inactivated vaccine** vaccine composed of whole pathogen cells or viruses that have been killed or inactivated through treatment with heat, radiation, or chemicals
- **incidence** the number of individuals with new infections of a particular disease in a given period of time
- **inclusion conjunctivitis** inflammation of the conjunctiva in newborns caused by *Chlamydia trachomatis* transmitted during childbirth
- **inclusions** prokaryotic cell cytoplasmic structures for storing specific nutrients and other resources needed by cells
- **incubation period** the first stage of acute disease, during which the pathogen begins multiplying in the host and signs and symptoms are not observable
- **indirect agglutination assay** assay that can be used to detect the agglutination of small latex beads; beads may be coated with antigen

when looking for the presence of specific antibodies, or with antibody when looking for the presence of antigen

- **indirect antiglobulin test (IAT)** see indirect Coombs' test
- **indirect contact transmission** transfer of an infectious agent between hosts through contact with a fomite
- **indirect Coombs' test** assay, performed *in vitro* prior to blood transfusions, that looks for antibodies against red blood cell antigens (other than the A and B antigens) that are unbound in a patient's serum
- **indirect ELISA EIA** in which an antigen from a pathogen is first attached to the wells of a microtiter plate; the antigen then captures antibodies from patient serum to determine whether the patient currently has or previously had the disease
- **indirect fluorescent antibody test** assay for antigen-specific antibodies wherein the antigen captures the antibody, which is subsequently detected using a labeled anti-immunoglobulin mAb
- **induced mutation** mutation caused by a mutagen
- **inducer** small molecule that either activates or represses transcription
- **inducible operon** bacterial operon, typically containing genes encoding enzymes in a degradative pathway, whose expression is induced by the substrate to be degraded when the substrate is available for the cell to use, but that is otherwise repressed in the absence of the substrate
- **induction** prophage DNA is excised from the bacterial genome
- **infection** the successful colonization of a microorganism within a host
- **infectious arthritis (septic arthritis)** inflammation of joint tissues in response to a microbial infection
- **infectious disease** disease caused by a pathogen
- **infectious mononucleosis** common and mild infection caused by Epstein-Barr virus (HHV-4) or cytomegalovirus (HHV-5); transmitted by direct contact with body fluids such as saliva
- **inflammation** innate nonspecific immune response characterized by erythema, edema, heat, pain, and altered function, typically at the site of injury or infection but sometimes becoming systemic.
- **influenza** highly contagious and acute viral disease of the respiratory tract caused by the influenza virus
- **initiation factors** proteins that participate in ribosome assembly during initiation

- **initiation of DNA replication** stage of replication during which various proteins bind to the origin of replication to begin the replication process
- **initiation of transcription** stage of transcription during which RNA polymerase binds to a promoter and transcription begins
- **initiation of translation** stage of translation during which an initiation complex composed of the small ribosomal subunit, the mRNA template, initiation factors, GTP, and a special initiator tRNA forms, and the large ribosomal subunit then binds to the initiation complex
- **inoculum** small number of cells added to medium to start a culture
- **inorganic phosphate (P_i)** single phosphate group in solution
- **insertion** type of mutation involving the addition of one or more bases into a DNA sequence
- **integrase inhibitors** antiviral drugs that block the activity of the HIV integrase responsible for recombination of a DNA copy of the viral genome into the host cell chromosome
- **intercalating agent** molecule that slides between the stacked nitrogenous bases of the DNA double helix, potentially resulting in a frameshift mutation
- **interference** distortion of a light wave due to interaction with another wave
- **interferons** cytokines released by cells that have been infected with a virus; stimulate antiviral responses in nearby cells as well as the cells secreting the interferons
- **interleukins** cytokines largely produced by immune system cells that help coordinate efforts against invading pathogens
- **intermediate filament** one of a diverse group of cytoskeletal fibers that act as cables within the cell and anchor the nucleus, comprise the nuclear lamina, or contribute to the formation of desmosomes
- **intermediate host** a host in which a parasite goes through some stages of its life cycle before migrating to the definitive host
- **intermittent common source spread** a mode of disease transmission in which every infection originates from the same source and that source produces infections for a period before stopping and then starting again
- **intertrigo** a rash that occurs in a skin fold

- **intestinal fluke** a trematode worm that infects the intestine, often caused by *Fasciolopsis buski*
- **intracellular targeting toxin** see *A-B exotoxin*
- **intrinsic growth rate** genetically determined generation time under specific conditions for a bacterial strain
- **intron** intervening sequence of a eukaryotic gene that does not code for protein and whose corresponding RNA sequences are removed from the primary transcript during splicing
- **intubation** placement of a tube into the trachea, generally to open the airway or to administer drugs or oxygen
- **in-use test** a technique for monitoring the correct use of disinfectants in a clinical setting; involves placing used, diluted disinfectant onto an agar plate to see if microbial colonies will grow
- **invasion** dissemination of a pathogen through local tissues or throughout the body
- **iodophor** compound in which iodine is complexed to an organic molecule, increasing the stability and efficacy of iodine as a disinfectant
- **ionizing radiation** high-energy form of radiation that is able to penetrate surfaces and sterilize materials by damaging microbial cell components and DNA
- **ischemia** condition marked by the inadequate flow of blood to the tissues
- **isograft** tissue grafted from one monozygotic twin to another
- **isoagglutinins** IgM class antibodies produced against A or B red blood cell antigens
- **isomers** molecules that have the same atomic makeup but differ in the structural arrangement of the atoms
- **isoniazid** antimetabolite that inhibits biosynthesis of mycolic acid; used for the treatment of mycobacterial infections
- **isoprenoid** branched lipid derived from five-carbon isoprene molecules
- **isotonic medium** a solution in which the solute concentrations inside and outside the cell are approximately equal, thereby creating no net movement of water molecules across the cell membrane
- **ivermectin** antihelminthic drug of the avermectin class that binds to invertebrate glutamate-gated chloride channels to block neuronal

transmission in helminths

J

- **Japanese encephalitis** arboviral disease caused by the Japanese encephalitis virus (JEV) and endemic to Asia
- **jaundice** yellowish color of the skin and mucous membranes caused by excessive bilirubin caused by a failure of the liver to effectively process the breakdown of hemoglobin

K

- **keratin** a fibrous protein found in hair, nails, and skin
- **keratitis** inflammation of the cornea
- **keratoconjunctivitis** inflammation of both the cornea and the conjunctiva
- **kidney** organ that filters the blood, producing urine
- **Kinyoun technique** a method of acid-fast staining that does not use heat to infuse the primary stain, carbolfuchsin, into acid-fast cells
- **Kirby-Bauer disk diffusion test** simple, rapid method for determining susceptibility and resistance of a bacterial pathogen to antibacterial drugs. The test involves drug-impregnated disks placed on an agar plate inoculated with a bacterial lawn.
- **Koplik's spots** white spots that form on the inner lining of the cheek of patients with measles
- **Krebs cycle** cyclic pathway during which each two-carbon unit entering the cycle is further oxidized, producing three NADH, one FADH₂, and one ATP by substrate-level phosphorylation, releasing two CO₂ molecules and regenerating the molecule used in the first step; also called the *citric acid cycle* or the *tricarboxylic acid cycle*
- **kuru** rare form of transmissible spongiform encephalopathy endemic to Papua New Guinea

L

- **lacrimal duct** connects the lacrimal gland to the lacrimal sac
- **lacrimal gland** a gland situated above the eye that secretes tears
- **lacrimal punctum** opening in each upper and lower eyelid
- **lacrimal sac** a to a reservoir for tears; also known as the dacrocyst or tear sac
- **lag period** the time between antigen exposure and production of antibodies
- **lag phase** interval before exponential growth of a microbial population during which cells adjust to a new environment
- **lagging strand** strand of DNA made discontinuously by DNA polymerase
- **laryngitis** inflammation of the larynx
- **laryngopharynx** lower portion of the pharynx that connects to the larynx
- **larynx** region of the respiratory tract containing the vocal cords; also referred to as the voice box
- **latent disease** disease that goes into a dormant nonreplicative state after the acute disease and can persist in this state for years, with the risk of reactivation back into acute disease
- **latent virus** virus that remains dormant in the host genome
- **lateral flow test** see immunochromatographic assays
- **leading strand** strand of DNA made continuously in the 5' to 3' direction by DNA polymerase
- **Legionnaires disease** atypical pneumonia occurring in older individuals; caused by the inhalation of *Legionella pneumophila* aerosolized in water
- **leishmaniasis** protozoan infection caused by *Leishmania* spp. and transmitted by sand flies
- **leprosy** see *Hansen's disease*
- **leptospirosis** bacterial infection of the kidney caused by *Leptospira* spp.; may spread to the liver, lungs, brain, and other organs
- **leukocidin** class of exotoxin that targets and lyses leukocytes
- **leukocytes** white blood cells of various types, including granulocytes, lymphocytes, and monocytes

- **leukotrienes** lipid-based chemical mediators produced by leukocytes and other tissue cells; promote inflammation and allergic responses
- **lichen** symbiotic association of a fungus with an algae or cyanobacterium
- **ligation** repair of the sugar-phosphate backbone of the DNA, making the DNA molecule continuous
- **light chains** the shorter identical peptide chains of an antibody molecule (two per antibody monomer), composed of variable and constant region segments
- **light-dependent reaction** process by which energy from sunlight is absorbed by pigment molecules in photosynthetic membranes and converted into stored chemical energy in the forms of ATP and NADPH
- **light-harvesting complex** group of multiple proteins and associated pigments that each may absorb light energy to become excited, and transfer this energy from one pigment molecule to another until the energy is delivered to a reaction center pigment
- **light-independent reaction** process by which chemical energy, in the form of ATP and NADPH produced by the light-dependent reactions, is used to fix inorganic CO₂ into organic sugar; usually referred to as the Calvin-Benson cycle
- **lincomycin** naturally produced protein synthesis inhibitor of the lincosamide class that binds to the 50S subunit, inhibiting peptide bond formation
- **lincosamides** class of protein synthesis inhibitors that are similar to macrolides
- **linked recognition** a mechanism whereby a B cell and the helper T cell with which it interacts recognize the same antigen
- **lipase** extracellular enzyme that degrades triglycerides
- **lipid bilayer** biological membranes composed of two layers of phospholipid molecules with the nonpolar tails associating to form a hydrophobic barrier between the polar heads; also called unit membrane
- **lipid** macromolecule composed primarily of carbon and hydrogen; source of nutrients for organisms, a storage form for carbon and energy, a part of the structure of membranes, and may function as hormones, pharmaceuticals, fragrances, and pigments

- **lipopolysaccharide (LPS)** lipid molecules with attached sugars that are found as components of gram-negative outer membranes
- **lipoprotein** conjugated protein attached to a lipid
- **listeriosis** bacterial disease caused from the ingestion of the microbe *Listeria monocytogenes*
- **lithotroph** chemotroph that uses inorganic chemicals as its electron source; also known as chemoautotroph
- **live attenuated vaccine** vaccine with live pathogen that has been attenuated to become less virulent in order to produce an active but subclinical infection
- **liver fluke** a trematode worm that affects the bile duct of the liver, including *Fasciola hepatica* and *F. gigantica*
- **local infection** infection in one limited area
- **log phase** interval of growth when cells divide exponentially; also known as the exponential growth phase
- **loiasis** a disease caused by the parasitic *Loa loa* worm, which is transmitted by deerflies; adult worms live in the subcutaneous tissue and cause inflammation, swelling, and eye pain as they migrate through the skin and the conjunctiva of the eye
- **lophotrichous** having a single tuft of flagella located at one end of a bacterial cell
- **low G+C gram-positive bacteria** bacteria that have less than 50% of guanine and cytosine nucleotides in their DNA
- **lumen** space inside the cisternae of the endoplasmic reticulum in eukaryotic cells
- **Lyme disease** tickborne disease caused by the spirochete *Borrelia burgdorferi*
- **lymph nodes** bean-shaped organs situated throughout the body that contain areas called germinal centers, which are rich in B and T lymphocytes; also contain macrophages and dendritic cells for antigen presentation
- **lymphadenitis** inflammation of the lymph nodes
- **lymphangitis** inflammation of the lymphatic vessels
- **lymphogranuloma venereum** infection caused by *Chlamydia trachomatis* in tropical regions
- **lyophilization** rapid freezing, followed by placement under a vacuum, of a material so that water is lost by sublimation, thereby inhibiting

microbial growth

- **lysis** destruction of the host cell
- **lysogen** bacterium carrying the prophage
- **lysogenic conversion (phage conversion)** alteration of host characteristics or phenotypes due to the presence of phage
- **lysogenic cycle** life cycle of some phages in which the genome of the infecting phage is integrated into the bacterial chromosome and replicated during bacterial reproduction until it excises and enters a lytic phase of the life cycle
- **lysogeny** process of integrating the phage into the host genome
- **lysosome** an organelle of the endomembrane system that contains digestive enzymes that break down engulfed material such as foodstuffs, infectious particles, or damaged cellular components
- **lytic cycle** infection process that leads to the lysis of host cells

M

- **M protein** a streptococcal cell wall protein that protects the bacteria from being phagocytized. It is associated with virulence and stimulates a strong immune response
- **macrolides** class of protein synthesis inhibitors containing a large, complex ring structure that binds to the 50S subunit, inhibiting peptide bond formation
- **macromolecule** polymer assembled from individual units, monomers, that bind together like building blocks
- **macronucleus** larger nucleus in ciliate protists that have two nuclei; polyploid with a reduced genome of metabolic genes and derived from the micronucleus
- **macronutrient** element required in abundance in cells; account for approximately 99% of the cell's dry weight
- **macrophages** monocytes that have left the bloodstream and differentiated into tissue-specific phagocytes
- **mad cow disease** form of transmissible spongiform encephalopathy primarily affecting cattle; can be transmitted to humans by consumption of contaminated cattle products

- **magnetosomes** inclusions in certain bacterial cells containing magnetic iron oxide or iron sulfide, which allows bacteria to align along a magnetic field by magnetotaxis
- **magnetotaxis** directional movement of bacterial cells using flagella in response to a magnetic field
- **magnification** the power of a microscope (or lens) to produce an image that appears larger than the actual specimen, expressed as a factor of the actual size
- **major histocompatibility complex (MHC)** collection of genes that code for MHC glycoproteins expressed on the surface of all nucleated cells
- **malaise** a general feeling of being unwell
- **malaria** potentially fatal, mosquito-borne protozoan infection caused by several species of *Plasmodium* and characterized by a relapsing fever, nausea, vomiting, and fatigue
- **mast cells** granulocytes similar in origin and function to basophils, but residing in tissues
- **matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF)** technique in which the sample (e.g., a microbe colony) is mixed with a special matrix and irradiated with a high-energy laser to generate characteristic gaseous ions that are subjected to mass spectral analysis, yielding mass spectra that may be compared to reference data for identification purposes
- **maturation** assembly of viral components to produce a functional virus
- **mature naïve T cell** a T cell that has exited the thymus after thymic selection but has not yet been activated
- **maximum growth pH** highest pH value that an organism can tolerate for growth
- **maximum growth temperature** highest temperature at which a microorganism will divide or survive
- **maximum permissible oxygen concentration** highest concentration of oxygen at which an organism will grow
- **measles** highly contagious respiratory disease caused by the measles virus (MeV); marked by an intense macular rash and high fever; also known as rubeola

- **mebendazole** antihelminthic drug of the benzimidazole class that binds to helminthic β -tubulin, preventing microtubule formation
- **mechanical transmission** transfer of a pathogen between hosts by a mechanical vector
- **mechanical vector** an animal that transfers a pathogen from one host to another or from a reservoir to a host without being infected by the pathogen itself
- **median infectious dose (ID_{50})** concentration of pathogen that will produce active infection in 50% of test animals inoculated
- **median lethal dose (LD_{50})** concentration of pathogen that kills 50% of infected test animals
- **medulla** loosely packed layer of fungal filaments located underneath the cortex of a lichen
- **membrane attack complex (MAC)** ring structure formed from complement proteins C6 through C9 that penetrates the membranes of a targeted cell, causing cell lysis and death
- **membrane filtration** method to remove bacteria from liquid, typically heat-sensitive solutions, using filters with an effective pore size of 0.2 μm or smaller, depending on need
- **membrane filtration technique** known volumes are vacuum filtered aseptically through a membrane with a pore size small enough to trap microorganisms, which are counted after growth on plates
- **membrane-bound ribosome** 80S eukaryotic ribosome attached to rough endoplasmic reticulum
- **membrane-disrupting toxin** toxin that affects cell membrane function by either forming pores or disrupting the phospholipid bilayer
- **memory B cell** an activated and differentiated B cell that is programmed to respond to secondary exposures to a specific antigen
- **memory helper T cell** a long-lived T cell programmed to recognize and quickly mount a secondary response to a specific pathogen upon re-exposure
- **memory** the ability of the specific adaptive immune system to quickly respond to pathogens to which it has previously been exposed
- **meninges** membranes that surround the brain
- **meningitis** inflammation of the meningeal membranes that surround the brain

- **meningococcal meningitis** bacterial infection caused by *Neisseria meningitidis* that results in an inflammation of the meninges
- **meningoencephalitis** inflammatory response that involves both the brain and the membranes that surround it
- **MERS** Middle East respiratory syndrome; first described in Saudi Arabia in 2013; caused by a zoonotic coronavirus that results in flu-like symptoms
- **mesophile** a microorganism that grows best at moderate temperatures, typically between about 20 °C and 45 °C
- **metabolism** all of the chemical reactions inside of cells
- **metachromatic granule** a type of inclusion containing volutin, a polymerized inorganic phosphate that appears red when stained with methylene blue
- **metagenomics** the sequencing of genomic fragments from microbial communities, allowing researchers to study genes from a collection of multiple species
- **metatranscriptomics** the science of studying a collection of mRNA molecules produced from microbial communities; involves studying gene expression patterns from a collection of multiple species
- **methanogen** microorganism that produces gaseous methane
- **methicillin-resistant *Staphylococcus aureus* (MRSA)** pathogen resistant to all β-lactams through acquisition of a new low-affinity penicillin-binding protein, and often resistant to many other drug classes
- **metronidazole** antibacterial and antiprotozoan drug of the nitroimidazole class that is activated in anaerobic target cell and introduces DNA strand breakage, thus interfering with DNA replication in target cells
- **MHC I molecule** glycoprotein expressed on the surface of all nucleated cells and involved in the presentation of normal “self” antigens and foreign antigens from intracellular pathogens
- **MHC II molecule** glycoprotein expressed only on the surface of antigen-presenting cells and involved in the presentation of foreign antigens from pathogens ingested by phagocytosis
- **micelle** simple spherical arrangement of amphipathic lipid molecules with nonpolar tails aggregated within the interior and polar heads forming the outer surface

- **microaerophile** organism that requires oxygen at levels lower than atmospheric concentration
- **microarray analysis** a technique used to compare two samples of genomic DNA or cDNA; the DNA or cDNA fragments are immobilized on a chip and labeled with different fluorescent dyes, allowing for comparison of sequences or gene-expression patterns
- **microbe** generally, an organism that is too small to be seen without a microscope; also known as a microorganism
- **microbial death curve** graphical representation of the progress of a particular microbial control protocol
- **microbial ecology** study of the interactions between microbial populations microbiology the study of microorganisms
- **microbiome** all prokaryotic and eukaryotic microorganisms that are associated with a certain organism
- **microfilament** cytoskeletal fiber composed of actin filaments
- **microinjection** the direct injection of DNA into the cytoplasm of a eukaryotic cell using a glass micropipette
- **micronucleus** smaller nucleus in ciliate protists that have two nuclei; diploid, somatic, and used for sexual reproduction through conjugation
- **micronutrient** indispensable element present in cells in lower amounts than macronutrients; also called *trace element*
- **microorganism** generally, an organism that is too small to be seen without a microscope; also known as a microbe
- **microsporidia** fungi that lack mitochondria, centrioles, and peroxisomes; some can be human pathogens
- **microtiter plates** plastic dishes with multiple small wells
- **microtubule** hollow tube composed of tubulin dimers (α and β tubulin); the structural component of the cytoskeleton, centrioles, flagella, and cilia
- **miliary tuberculosis** hematogenous dissemination and spread of *Mycobacterium tuberculosis* from tubercles
- **minimal bactericidal concentration (MBC)** lowest antibacterial drug concentration that kills $\geq 99.9\%$ of a starting inoculum of bacteria
- **minimal inhibitory concentration (MIC)** lowest concentration of an antibacterial drug that inhibits visible growth of a bacterial strain
- **minimum growth pH** lowest pH value that an organism can tolerate for growth

- **minimum growth temperature** lowest temperature at which a microorganism will divide or survive
- **minimum permissible oxygen concentration** lowest concentration of oxygen at which an organism will grow
- **missense mutation** point mutation that results in a different amino acid being incorporated into the resulting polypeptide
- **mitochondrial matrix** the innermost space of the mitochondrion enclosed by two membranes; the location of many metabolic enzymes as well as the mitochondrial DNA and 70S ribosomes
- **mitochondrion (plural: mitochondria)** large, complex organelle that is the site of cellular respiration in eukaryotic cells
- **mode of action** way in which a drug affects a microbe at the cellular level
- **moist-heat sterilization** protocol that involves steam under pressure in an autoclave, allowing the steam to reach temperatures higher than the boiling point of water
- **mold** a multicellular fungus, typically made up of long filaments
- **molecular cloning** the purposeful fragmentation of DNA followed by attachment to another piece of DNA to produce a recombinant molecule, followed by introduction of this recombinant molecule into an easily manipulated host to allow for the creation of multiple copies of a gene of interest
- **monoclonal antibodies (mAbs)** antibodies produced *in vitro* that only bind to a single epitope
- **monocular** having a single eyepiece
- **monocytes** large, agranular, mononuclear leukocytes found in the peripheral blood; responsible for phagocytosis of pathogens and damaged cells
- **monoecious** refers to sexually reproducing organisms in which individuals have both male and female reproductive organs
- **monomer** small organic molecule that binds with like molecules, forming a polymer or macromolecule
- **monosaccharide** monomer for the synthesis of carbohydrate polymers; the simplest carbohydrate, called a *simple sugar*
- **monotrichous** having one flagellum, typically located on one end of the bacterial cell
- **morbidity** a state of illness

- **Morbidity and Mortality Weekly Report (MMWR)** the trade/industry publication for epidemiologists, reporting US public health data compiled by the CDC
- **morbidity rate** the number of cases of a disease expressed as a percentage of the population or number per standard part of the population, such as 100,000
- **mordant** a chemical added to a specimen that sets a stain
- **mortality** death
- **mortality rate** the number of deaths from a disease expressed as a percentage of the population or number per standard part of the population, such as 100,000
- **most probable number (MPN)** statistical value representing the viable bacterial population in a sample obtained after a series of dilutions and multiple tube inoculations
- **mRNA** short-lived type of RNA that serves as the intermediary between DNA and the synthesis of protein products
- **mucociliary escalator** system by which mucus and debris are propelled up and out of the respiratory tract by the beating of respiratory cilia and the mechanical actions of coughing or swallowing
- **mucormycosis** rare form of pneumonia that can be caused by an invasive infection of different fungi in the order Mucorales, such as *Rhizopus* or *Mucor*
- **mucous membrane** moist layer of epithelial cells and interspersed goblet cells that lines the inner surfaces of the body, usually bathed in antimicrobial secretions from the cells of the membrane
- **mucus** viscous secretion produced by cells and glands in various mucous membranes throughout the body; helps trap and remove microbes and debris from the body
- **multidrug-resistant microbes (MDR)** group of pathogens that carry one or more resistance mechanisms, making them resistant to multiple antimicrobials; also called superbugs
- **multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB)** strains of *M. tuberculosis* that are resistant to both rifampin and isoniazid, the drug combination typically prescribed for the treatment of tuberculosis
- **multiple sclerosis** autoimmune attack on the myelin sheaths and nerve cells in the central nervous system

- **mumps** a viral illness that causes swelling of the parotid glands; rare in the United States because of effective vaccination
- **murine typhus** fleaborne infection caused by *Rickettsia typhi* and characterized by fever, rash, and pneumonia
- **mutagen** type of chemical agent or radiation that can induce mutations
- **mutant** organism harboring a mutation that often has a recognizable change in phenotype compared to the wild type
- **mutation** heritable change in the DNA sequence of an organism
- **mutualism** type of symbiosis in which two populations benefit from, and depend on, each other
- **myasthenia gravis** autoimmune disease affecting the acetylcholine receptors in the neuromuscular junction, resulting in weakened muscle contraction capability
- **mycelium** vegetative network of branched, tubular hyphae
- **mycolic acids** waxy molecules associated with peptidoglycan in some gram-positive, acid-fast bacteria, chiefly mycobacteria
- **mycology** the study of fungi
- ***Mycoplasma pneumonia*** also known as walking pneumonia; a milder form of atypical pneumonia caused by *Mycoplasma pneumoniae*
- **mycoses (mycosis, sing.)** refers to diseases caused by fungi
- **mycotoxin** biologically active product of pathogenic fungi that causes adverse changes in the host cells
- **myelin sheath** insulating layer that surrounds the axon of some neurons and helps to promote signal propagation
- **myocarditis** inflammation of the heart muscle tissues

N

- **naïve mature B cell** a B cell that has not yet been activated
- **naked virus** virus composed of a nucleic acid core, either DNA or RNA, surrounded by a capsid
- **nalidixic acid** member of the quinolone family that functions by inhibiting the activity of DNA gyrase, blocking DNA replication
- **narrow-spectrum antimicrobial** drug that targets only a specific subset of microbes
- **nasal cavity** air-filled space in the skull immediately behind the nose

- **nasolacrimal duct** tear duct connecting the lacrimal glands to the nasal cavity
- **nasolacrimal duct** tear fluid flows from each eye through this duct to the inner nose
- **nasopharynx** part of the upper throat (pharynx) extending from the posterior nasal cavity; carries air inhaled through the nose
- **native structure** three-dimensional structure of folded fully functional proteins
- **natural active immunity** immunity that develops as a result of natural infection with a pathogen
- **natural antibiotic** antimicrobial compound that is produced naturally by microorganisms in nature
- **natural killer cells (NK cells)** lymphoid cells that recognize and destroy abnormal target cells by inducing apoptosis
- **natural passive immunity** transfer of maternal antibodies from mother to fetus (transplacentally) or infant (via breastmilk)
- **necrotizing fasciitis** a serious infection, also known as flesh-eating disease, that leads to rapid destruction of tissue through the action of exotoxin A; it can be caused by *S. pyogenes* or several other bacterial species
- **negative (-) single-strand RNA (-ssRNA)** a viral RNA strand that cannot be translated until it is replicated into positive single-strand RNA by viral RNA-dependent RNA polymerase
- **negative stain** a stain that produces color around the structure of interest while not coloring the structure itself
- **Nematoda** phylum comprising roundworms
- **neonatal herpes** herpes infection of the newborn, generally caused by infection during birth
- **neonatal meningitis** meningitis caused by Group B streptococcus and occurring primarily in neonates (less than 2 months old)
- **neonatal tetanus** tetanus acquired through infection of the cut umbilical cord
- **neurocysticercosis** parasitic invasion of brain tissues by the larvae of the pork tapeworm, *Taenia solium*
- **neuromycosis** any fungal infection of the nervous system
- **neuron** specialized cell found throughout the nervous system that transmits signals through the nervous system using electrochemical

processes

- **neuropathy** numbness or tingling sensation caused by damage to peripheral nerves
- **neurotoxoplasmosis** disease caused by the invasion of brain tissues by the protozoan *Toxoplasma gondii*; typically only affects immunocompromised patients
- **neurotransmitter** compound that is released at the synapse of neurons to stimulate or suppress the actions of other cells
- **neutralism** type of symbiosis that does not affect either of the two populations
- **neutralization** binding of an antibody to a pathogen or toxin, preventing attachment to target cells
- **neutrophile** organism that grows best at a near a neutral pH of 6.5–7.5
- **neutrophils** leukocytes with a multilobed nucleus found in large numbers in peripheral blood; able to leave the bloodstream to phagocytose pathogens in infected tissues; also called polymorphonuclear neutrophils (PMNs)
- **next generation sequencing** a group of automated techniques used for rapid DNA sequencing
- **nicotine adenine dinucleotide (NAD⁺/NADH)** oxidized/reduced forms of an electron carrier in cells
- **nicotine adenine dinucleotide phosphate (NADP⁺/NADPH)** oxidized/reduced forms of an electron carrier in cells
- **nitrogen fixation** bacterial biochemical pathways that incorporate inorganic nitrogen gas into organic forms more easily used by other organisms
- **nitrogenous base** nitrogen-containing ring structure within a nucleotide that is responsible for complementary base pairing between nucleic acid strands
- **noncoding DNA** regions of an organism's genome that, unlike genes, do not encode proteins
- **noncommunicable disease** disease that is not transmitted from one person to another
- **noncompetitive (allosteric) inhibitor** molecule that binds to allosteric sites, inducing a conformational change in the enzyme's structure that prevents it from functioning

- **noncritical item** object that may contact intact skin but does not penetrate it; requires cleanliness but not a high level of disinfection
- **noncyclic photophosphorylation** pathway used in photosynthetic organisms when both ATP and NADPH are required by the cell
- **nonenveloped virus** naked virus
- **nongonococcal urethritis (NGU)** a nonspecific infection of the urethra that is not caused by *Neisseria gonorrhoeae*
- **noninfectious disease** disease caused by something other than an infectious agent (e.g., genetics, environment, nutritional deficiencies)
- **nonionizing radiation** low-energy radiation, like ultraviolet light, that can induce dimer formation between two adjacent pyrimidine bases, resulting in DNA polymerase stalling and possible formation of a frameshift mutation
- **nonsense mutation** point mutation that converts a codon encoding an amino acid (a sense codon) into a stop codon (a nonsense codon)
- **nontreponemal serologic tests** qualitative and quantitative indirect diagnostic tests for syphilis
- **northern blot** a technique in molecular genetics used to detect the amount of RNA made by gene expression within a tissue or organism sample; RNA fragments within a sample are separated by agarose gel electrophoresis, immobilized on a membrane, and then exposed to a specific DNA probe labeled with a radioactive or fluorescent molecular beacon to aid in detection
- **nosocomial disease** disease acquired in a hospital setting
- **notifiable disease** a disease for which all cases must legally be reported to regional, state, and/or federal public health agencies
- **nuclear envelope** (also called the nuclear membrane) a structure defining the boundary of the nucleus; composed of two distinct lipid bilayers that are contiguous with each other and with the endoplasmic reticulum
- **nuclear lamina** a meshwork of intermediate filaments (mainly lamins) found just inside the nuclear envelope; provides structural support to the nucleus
- **nucleic acid** class of macromolecules composed of nucleotide monomers polymerized into strands
- **nucleoid** concentrated area of DNA genome and associated proteins found in a prokaryotic cell that is not surrounded by a membrane

- **nucleoid-associated protein (NAP)** protein that assists in the organization and packaging of the chromosome in prokaryotic cells
- **nucleolus** a dense region within the nucleus where ribosomal RNA biosynthesis occurs and preribosomal complexes are made
- **nucleoside analog** chemical that is structurally similar to a normal nucleotide base that can be incorporated into DNA instead of normal bases during replication but that has different base pairing rules than the normal base for which it was substituted, inducing mutation
- **nucleotide excision repair (dark repair)** enzymatic mechanism to repair pyrimidine dimers by cutting the dimer-containing DNA strand on both sides of dimer, removing the intervening strand and replacing the bases with the correct ones
- **nucleotide** nucleic acid monomer composed of a pentose sugar, a phosphate group, and a nitrogenous base
- **nucleus** a membrane-bound structure of eukaryotic cells that houses the DNA genome
- **numerical aperture** a measure of a lens's ability to gather light

O

- **objective lenses** on a light microscope, the lenses closest to the specimen, typically located at the ends of turrets
- **obligate aerobe** organism that requires oxygen for growth
- **obligate anaerobe** organism that dies in the presence of oxygen
- **obligate intracellular pathogen** microorganism that cannot synthesize its own ATP and, therefore, must rely on a host cell for energy; behaves like a parasite when inside a host cell, but is metabolically inactive outside of a host cell
- **observational study** a type of scientific study that involves measurement of study subjects on variables hypothesized to be associated with the outcome of interest, but without any manipulation of the subjects
- **ocular lens** on a microscope, the lens closest to the eye (also called an eyepiece)
- **oil immersion lens** a special objective lens on a microscope designed to be used with immersion oil to improve resolution

- **Okazaki fragment** short fragment of DNA made during lagging strand synthesis
- **oligopeptide** peptide having up to approximately 20 amino acids
- **oligotroph** organism capable of living in low-nutrient environments
- **opacity** the property of absorbing or blocking light
- **operator** DNA sequence located between the promoter region and the first coding gene to which a repressor protein can bind
- **operon** a group of genes with related functions often found clustered together within the prokaryotic chromosome and transcribed under the control of a single promoter and operator repression sequence
- **ophthalmia neonatorum** inflammation of the conjunctiva in newborns caused by *Neisseria gonorrhoeae* transmitted during childbirth
- **opisthotonus** characteristic symptom of tetanus that results in uncontrolled muscular spasms and backward arching of the neck and spine
- **opportunistic pathogen** microorganism that can cause disease in individuals with compromised host defenses
- **opsonin** any molecule that binds to and coats the outside of a pathogen, identifying it for destruction by phagocytes (examples include antibodies and the complement proteins C3b and C4b)
- **opsonization** process of coating a pathogen with a chemical substance (an opsonin) that allows phagocytic cells to recognize, engulf, and destroy the pathogen more easily
- **optimum growth pH** the pH at which an organism grows best
- **optimum growth temperature** the temperature at which a microorganism's growth rate is highest
- **optimum oxygen concentration** the ideal concentration of oxygen for a particular microorganism
- **oral herpes** an infection caused by herpes simplex virus that results in cold sores, most commonly on and around the lips
- **oral thrush** *Candida* infection of the mouth
- **orchitis** inflammation of one or both of the testes
- **organic molecule** composed primarily of carbon; typically contains at least one carbon atom bound to one or more hydrogen atoms
- **organotroph** chemotroph that uses organic molecules as its electron source; also known as chemoheterotroph

- **origin of replication** specific nucleotide sequence where replication begins
- **oropharynx** area where air entering mouth enters the pharynx
- **osmosis** diffusion of water across a semipermeable membrane
- **osmotic pressure** the force or pressure generated by water diffusing across a semipermeable membrane, driven by differences in solute concentration across the membrane
- **osteomyelitis** inflammation of bone tissue
- **otitis externa** an infection of the external ear canal, most commonly caused by *Pseudomonas aeruginosa*; often called swimmer's ear
- **otitis** inflammation of the ear
- **otitis media with effusion** accumulation of fluid inside the middle ear with or without infection
- **Ouchterlony assay** test in which antigen and antisera are added to neighboring wells in an agar gel, allowing visualization of precipitin arcs
- **outer membrane** a phospholipid bilayer external to the peptidoglycan layer found in gram-negative cell walls
- **oxazolidinones** class of synthetic protein synthesis inhibitors that interfere with formation of the initiation complex for translation and prevent translocation of the growing protein from the ribosomal A site to the P site
- **oxidation reaction** chemical reaction that removes electrons (often as part of H atoms) from donor molecules, leaving them oxidized
- **oxidative phosphorylation** mechanism for making ATP that uses the potential energy stored within an electrochemical gradient to add P_i to ADP
- **oxygenic photosynthesis** type of photosynthesis found in plants, algae, and cyanobacteria, and in which H_2O is used as the electron donor to replace an electron lost by a reaction center pigment, resulting in oxygen as a byproduct

P

- **P (peptidyl) site** functional site of an intact ribosome that binds charged tRNAs carrying amino acids that have formed peptide bonds

with the growing polypeptide chain but have not yet dissociated from their corresponding tRNA

- **palatine tonsil** lymphoid tissue located near the oropharynx
- **pandemic disease** an epidemic that is worldwide as opposed to regional
- **papilloma** growth on the skin associated with infection by any of the human papilloma viruses (HPV); commonly known as a wart
- **paracrine function** refers to a cytokine signal released from a cell to a receptor on a nearby cell
- **parasitism** type of symbiosis in which one population benefits while harming the other parasitology the study of parasites
- **parenteral route** means of entry by a pathogen through skin or mucous membranes when these barriers are breached
- **paroxysmal stage** most serious stage of pertussis (whooping cough), characterized by severe and prolonged coughing spells
- **passive carrier** an individual capable of transmitting a pathogen to another individual without becoming infected
- **passive immunity** adaptive immune defenses received from another individual or animal
- **pasteurization** form of microbial control using heat that is applied to foods; kills pathogens and reduces the number of spoilage-causing microbes while maintaining food quality
- **pathogen** a disease-causing microorganism
- **pathogen-associated molecular patterns (PAMPs)** common molecular motifs found on pathogens
- **pathogenicity** ability of a microbial agent to cause disease
- **pattern recognition receptors (PRRs)** receptors on the surface or in the interior of phagocytic cells that bind to pathogen-associated molecular patterns (PAMPs)
- **pellicle** structure that underlies the plasma membrane in protists, providing additional support
- **pelvic inflammatory disease (PID)** infection of the female reproductive organs that may spread from the vagina to the cervix, uterus, fallopian tubes, and ovaries
- **penetration** entry of phage or virus into a host cell through injection, endocytosis, or membrane fusion

- **penicillin** β -lactam antibacterial that was the first cell wall synthesis inhibitor developed
- **penis** external genital organ in males through which urine and semen are discharged
- **pentamidine** antiprotozoan drug that appears to degrade kDNA in target cells, as well as inhibit protein synthesis
- **pentose phosphate pathway (PPP)** alternative glycolytic pathway that produces intermediates used for the biosynthesis of nucleotides and amino acids; also called the *phosphogluconate pathway* or the *hexose monophosphate shunt*
- **peptic ulcer** an ulcer in the lining of the stomach or duodenum, often associated with *Helicobacter pylori*
- **peptide bond** bond between the carboxyl group of one amino acid and the amine group of another; formed with the loss of a water molecule
- **peptidoglycan (murein)** the polymer of alternating N-acetylmuramic acid NAM and N-acetylglucosamine (NAG) subunits linked together by peptide chains; a major constituent of bacterial cell walls
- **peptidyl transferase** RNA-based ribozyme that is part of the 50S ribosomal subunit and catalyzes formation of the peptide bond between the amino acid bound to a tRNA and the growing polypeptide chain
- **perforin** compound released from a natural killer cell that creates pores in the target cell through which other toxins (particularly granzymes) can gain access to the cytoplasm
- **pericarditis** inflammation of the sac that surrounds the heart
- **period of convalescence** fifth stage of acute disease, during which the patient returns to normal function
- **period of decline** fourth stage of disease, during which the number of pathogens present in the host decreases, along with signs and symptoms of disease
- **period of illness** third stage of acute disease, during which the number of pathogens present in the host is greatest and the signs and symptoms of disease are most severe
- **periodontal disease** a condition in which the gums are inflamed and may erode
- **periodontitis** inflammation of the gums that is more severe than gingivitis, spreading deeper into the tissues

- **peripheral nervous system** network of neurons that connects the CNS with organs, sensory organs, and muscles throughout the body
- **peripheral tolerance** mechanism by which regulatory T cells inhibit self-reactive immune responses in T cells that have already exited the thymus
- **periplasmic space** the space between the cell wall and the plasma membrane, primarily in gram-negative bacteria
- **peristalsis** muscular contractions of the gastrointestinal tract that propel ingested material through the stomach, intestines, and, eventually, through the rectum and out of the body
- **peritrichous** having numerous flagella covering the entire surface of a bacterial cell
- **peroxidase** enzyme that catalyzes the detoxification of peroxides
- **peroxisome** in eukaryotic cells, a membrane-bound organelle (not part of the endomembrane system) that produces hydrogen peroxide to break down various types of molecules; also plays a role in lipid biosynthesis
- **peroxygent** type of strong oxidizing agent that causes free radical formation in cells; can be used as a disinfectant or antiseptic
- **persister** dormant cell that survives in the death phase and is resistant to most antibiotics
- **pertussis** contagious illness caused by *Bordetella pertussis* that causes severe coughing fits followed by a whooping sound during inhalation; commonly known as whooping cough
- **pertussis toxin** main virulence factor accounting for the symptoms of whooping cough
- **petechiae** small red or purple spots on the skin that result from blood leaking out of damaged vessels
- **Petroff-Hausser counting chamber** calibrated slide that allows counting of bacteria in a specific volume under a microscope
- **Peyer's patches** lymphoid tissue in the ileum that monitors and fights infections
- **phagemid** a plasmid capable of being replicated as a plasmid and also incorporated into a phage head
- **phagocytosis** a type of endocytosis in which large particles are engulfed by membrane invagination, after which the particles are

enclosed in a pocket, which is pinched off from the membrane to form a vacuole

- **phagolysosome** compartment in a phagocytic cell that results when the phagosome is fused with the lysosome, leading to the destruction of the pathogens inside
- **phagosome** compartment in the cytoplasm of a phagocytic cell that contains the phagocytosed pathogen enclosed by part of the cell membrane
- **pharmacogenomics (toxicogenomics)** the evaluation of the effectiveness and safety of drugs on the basis of information from an individual's genomic sequence as well as examination of changes in gene expression in response to the drug
- **pharyngitis** inflammation of the pharynx
- **pharynx** region connecting the nose and mouth to the larynx: the throat
- **phase-contrast microscope** a light microscope that uses an annular stop and annular plate to increase contrast
- **phenol coefficient** measure of the effectiveness of a chemical agent through comparison with that of phenol on *Staphylococcus aureus* and *Salmonella enterica* serovar Typhi
- **phenolics** class of chemical disinfectants and antiseptics characterized by a phenol group that denatures proteins and disrupts membranes
- **phenotype** observable characteristics of a cell or organism
- **phosphodiester bonds** linkage whereby the phosphate group attached to the 5' carbon of the sugar of one nucleotide bonds to the hydroxyl group of the 3' carbon of the sugar of the next nucleotide
- **phosphogluconate pathway** see *pentose phosphate pathway*
- **phospholipase** enzyme that degrades phospholipids
- **phospholipid** complex lipid that contains a phosphate group
- **phospholipid-derived fatty acids (PLFA) analysis** technique in which membrane phospholipids are saponified to release the fatty acids of the phospholipids, which can be subjected to FAME analysis for identification purposes
- **phosphorescence** the ability of certain materials to absorb energy and then release that energy as light after a delay
- **photosynthesis** process whereby phototrophic organisms convert solar energy into chemical energy that can then be used to build

carbohydrates

- **photosynthetic pigment** pigment molecule used by a cell to absorb solar energy; each one appears the color of light that it transmits or reflects
- **photosystem** organized unit of pigments found within a photosynthetic membrane, containing both a light-harvesting complex and a reaction center
- **phototaxis** directional movement using flagella in response to light
- **phototroph** organism that gets its energy from light
- **phototrophic bacteria** nontaxonomic group of bacteria that use sunlight as their primary source of energy
- **phylogeny** the evolutionary history of a group of organisms
- **phytoplankton** photosynthetic plankton
- **pia mater** fragile and innermost membrane layer surrounding the brain
- **pili** long protein extensions on the surface of some bacterial cells; specialized F or sex pilus aids in DNA transfer between cells
- **pinocytosis** a type of endocytosis in which small dissolved materials are endocytosed into smaller vesicles
- **plague** infectious epidemic disease caused by *Yersinia pestis*
- **plankton** microscopic organisms that float in the water and are carried by currents; they may be autotrophic (phytoplankton) or heterotrophic (zooplankton)
- **planktonic** free-floating or drifting in suspension
- **plantibodies** monoclonal antibodies produced in plants that are genetically engineered to express mouse or human antibodies
- **plaque** clear area on bacterial lawn caused by viral lysis of host cells
- **plasma cell** activated and differentiated B cell that produces and secretes antibodies
- **plasma** fluid portion of the blood that contains all clotting factors
- **plasma membrane** (also called the cell membrane or cytoplasmic membrane) lipid bilayer with embedded proteins that defines the boundary of the cell
- **plasmalemma** protist plasma membrane
- **plasmid** small, circular, double-stranded DNA molecule that is typically independent from the bacterial chromosome
- **plasmolysis** the separation of the plasma membrane away from the cell wall when a cell is exposed to a hypertonic environment

- **platelets** cell fragments in the peripheral blood that originate from megakaryocyte cells in the bone marrow; also called thrombocytes
- **Platyhelminthes** phylum comprising flatworms
- **pleconaril** an antiviral drug targeting picornaviruses that prevents the uncoating of virus particles upon their infection of host cells
- **pleomorphic** able to change shape
- **pneumococcal meningitis** bacterial infection caused by *Streptococcus pneumoniae* that results in an inflammation of the meninges
- **Pneumocystis pneumonia** common pulmonary infection in patients with AIDS; caused by *P. jirovecii*
- **pneumonia** pulmonary inflammation that causes the lungs to fill with fluids
- **pneumonic plague** rare form of plague that causes massive hemorrhages in the lungs and is communicable through aerosols
- **point mutation** mutation, most commonly a base substitution, that affects a single base pair
- **point source spread** a form of common source spread in which the transmission of a disease from the source occurs for a brief period that is less than the pathogen's incubation period
- **polar tubule** a tube-like structure produced by spores of parasitic Microsporidia fungi that pierces host cell membranes
- **poliomyelitis (polio)** disease caused by an infection of the enteric polio virus characterized by inflammation of the motor neurons of the brain stem and spinal cord; can result in paralysis
- **poly-A tail** string of approximately 200 adenine nucleotides added to the 3' end of a eukaryotic primary mRNA transcript to stabilize it
- **polyacrylamide gel electrophoresis (PAGE)** a method for separating populations of proteins and DNA fragments during Sanger sequencing of varying sizes by differential migration rates caused by a voltage gradient through a vertical gel matrix
- **polycistronic mRNA** single mRNA molecule commonly produced during prokaryotic transcription that carries information encoding multiple polypeptides
- **polyclonal antibodies** antibodies produced in a normal immune response, in which multiple clones of B cells respond to many different epitopes on an antigen

- **polyenes** class of antifungal drugs that bind to ergosterol to form membrane pores, disrupting fungal cell membrane integrity
- **polyhedral virus** virus with a three-dimensional shape with many facets
- **polyhydroxybutyrate (PHB)** a type of cellular inclusion surrounded by a phospholipid monolayer embedded with protein
- **polylinker site or multiple cloning site (MCS)** a short sequence containing multiple unique restriction enzyme recognition sites that are used for inserting foreign DNA into the plasmid after restriction digestion of both the foreign DNA and the plasmid
- **polymer** macromolecule composed of individual units, monomers, that bind together like building blocks.
- **polymerase chain reaction (PCR)** an *in vitro* molecular technique that rapidly amplifies the number of copies of specific DNA sequences to make the amplified DNA available for other analyses
- **polymorphonuclear neutrophil (PMN)** see *neutrophils*
- **polymyxins** lipophilic polypeptide antibiotics that target the lipopolysaccharide component of gram-negative bacteria and ultimately disrupt the integrity of their outer and inner membranes
- **polypeptide** polymer having from approximately 20 to 50 amino acids
- **polyphyletic** refers to a grouping of organisms that is not descended from a single common ancestor
- **polyribosome (polysome)** structure including an mRNA molecule that is being translated by multiple ribosomes concurrently
- **polysaccharide** polymer composed of hundreds of monosaccharides linked together by glycosidic bonds; also called *glycans*
- **portal of entry** anatomical feature of the body through which pathogens can enter host tissue
- **portal of exit** anatomical feature of the body through which pathogens can leave diseased individual
- **positive (+) strand** viral RNA strand that acts like messenger RNA and can be directly translated inside the host cell
- **positive stain** a stain that colors the structure of interest
- **pour plate method** a technique used for inoculating plates with diluted bacterial samples for the purpose of cell counting; cells are mixed with warm liquid agar before being poured into Petri dishes

- **praziquantel** antihelminthic drug that induces a calcium influx into tapeworms, leading to spasm and paralysis
- **precipitin** complex lattice of antibody and antigen that becomes too large to stay in solution
- **precipitin ring test** assay in which layers of antisera and antigen in a test tube form precipitin at the interface of the two solutions
- **prevalence** the total number or proportion of individuals in a population ill with a specific disease
- **primary amoebic meningoencephalitis (PAM)** acute and deadly parasitic infection of brain tissues by the amoeba *Naegleria fowleri*
- **primary antibody** in a sandwich ELISA, the antibody that is attached to wells of a microtiter plate to capture antigen from a solution, or in an indirect ELISA, the antigen-specific antibody present in a patient's serum
- **primary cell culture** cells taken directly from an animal or plant and cultured in vitro
- **primary immunodeficiency** genetic condition that results in impaired immune function
- **primary infection** initial infection produced by a pathogen
- **primary lymphoid tissue** one of two types of lymphatic tissue; comprises bone marrow and the thymus
- **primary pathogen** microorganism that can cause disease in the host regardless of the effectiveness of the host's immune system
- **primary response** the adaptive immune response produced upon first exposure to a specific antigen
- **primary stain** refers, in differential staining techniques, to the first dye added to the specimen
- **primary structure** bonding sequence of amino acids in a polypeptide chain **protein** macromolecule that results when the number of amino acids linked together becomes very large, or when multiple polypeptides are used as building subunits
- **primary transcript** RNA molecule directly synthesized by RNA polymerase in eukaryotes before undergoing the additional processing required to become a mature mRNA molecule
- **primase** RNA polymerase enzyme that synthesizes the RNA primer required to initiate DNA synthesis

- **primer** short complementary sequence of five to 10 RNA nucleotides synthesized on the template strand by primase that provides a free 3'-OH group to which DNA polymerase can add DNA nucleotides
- **prion** acellular infectious particle consisting of just proteins that can cause progressive diseases in animals and humans
- **prodromal period** second stage of acute disease, during which the pathogen continues to multiply in the host and nonspecific signs and symptoms become observable
- **progeny virus** newly assembled virions ready for release outside the cell
- **proglottid** body segment of a cestode (tapeworm)
- **prokaryote** an organism whose cell structure does not include a membrane-bound nucleus
- **prokaryotic cell** a cell lacking a nucleus bound by a complex nuclear membrane
- **promoter** DNA sequence onto which the transcription machinery binds to initiate transcription
- **propagated spread** the progression of an infectious disease from person to person, either indirectly or directly, through a population of susceptible individuals as one infected individual transmits the agent to others, who transmit it to others yet again
- **prophage** phage genome that has incorporated into the host genome
- **prospective study** a research design that follows cases from the beginning of the study through time to associate measured variables with outcomes
- **prostate gland** gland that contributes fluid to semen
- **prostatitis** inflammation of the prostate gland
- **protease** enzyme involved in protein catabolism that removes individual amino acids from the ends of peptide chains
- **protease inhibitor** class of antiviral drugs, used in HIV therapy and hepatitis C therapy, that inhibits viral-specific proteases, preventing viral maturation
- **protein signature** an array of proteins expressed by a cell or tissue under a specific condition
- **Proteobacteria** phylum of gram-negative bacteria
- **proteomic analysis** study of all accumulated proteins of an organism

- **proteomics** the study of the entire complement of proteins in an organism; involves monitoring differences in gene expression patterns between cells at the protein level
- **protists** informal name for diverse group of eukaryotic organisms, including unicellular, colonial, and multicellular types that lack specialized tissues
- **proton motive force** electrochemical gradient formed by the accumulation of hydrogen ions (also known as protons) on one side of a membrane relative to the other protozoan (plural: protozoa) a unicellular eukaryotic organism, usually motile
- **protozoans** informal term for some protists, generally those that are nonphotosynthetic, unicellular, and motile protozoology the study of protozoa
- **provirus** animal virus genome that has integrated into the host chromosome
- **pseudohyphae** short chains of yeast cells stuck together
- **pseudomembrane** grayish layer of dead cells, pus, fibrin, red blood cells, and bacteria that forms on mucous membranes of the nasal cavity, tonsils, pharynx, and larynx of individuals with diphtheria
- **pseudomembranous colitis** inflammation of the large intestine with the formation of a pseudomembrane; caused by *C. difficile*
- **pseudopodia** temporary projections involved in amoeboid movement; these “false feet” form by gel-sol cycling of actin polymerization/depolymerization
- **psittacosis** zoonotic *Chlamydophila* infection from birds that causes a rare form of pneumonia
- **psoriasis** autoimmune disease involving inflammatory reactions in and thickening of skin
- **psychrophile** a microorganism that grows best at cold temperatures; most have an optimum growth temperature of about 15 °C and can survive temperatures below 0 °C; most cannot survive temperatures above 20 °C
- **psychrotroph** a microorganism that grows best at cool temperatures, typically between about 4 °C and 25 °C, with optimum growth at about 20 °C
- **puerperal sepsis** sepsis associated with a bacterial infection incurred by a woman during or after childbirth

- **purines** nitrogenous bases containing a double-ring structure with a six-carbon ring fused to a five-carbon ring; includes adenine and guanine
- **purple nonsulfur bacteria** phototrophic bacteria that are similar to purple sulfur bacteria except they use hydrogen rather than hydrogen sulfide for oxidation
- **purple sulfur bacteria** phototrophic bacteria that oxidize hydrogen sulfide into elemental sulfur and sulfuric acid; their purple color is due to the pigments bacteriochlorophylls and carotenoids
- **purulent** an infection that produces pus; suppurative
- **pus** accumulation of dead pathogens, neutrophils, tissue fluid, and other bystander cells that may have been killed by phagocytes at the site of an infection
- **pyelonephritis** an infection of one or both kidneys
- **pyocyanin** blue pigments produced by some strains of *Pseudomonas aeruginosa*
- **pyoderma** any suppurative (pus-producing) infection of the skin
- **pyoverdin** a water-soluble, yellow-green or yellow-brown pigment produced by some strains of *Pseudomonas aeruginosa*
- **pyrimidines** nitrogenous bases containing a single six-carbon ring; includes cytosine and thymine in DNA
- **pyrophosphate (PPi)** two connected phosphate groups in solution
- **pyuria** pus or white blood cells in the urine

Q

- **Q fever** highly infectious zoonotic disease caused by *Coxiella burnetii* that farmers can contract from their animals by inhalation
- **quarantine** the isolation of an individual for the purpose of preventing the spread of disease
- **quaternary ammonium salts (quats)** group of cationic detergents, named for the characteristic quaternary nitrogen atom that confers a positive charge, that make up an important class of disinfectants and antiseptics
- **quaternary structure** structure of protein complexes formed by the combination of several separate polypeptides or subunits

- **quinolines** class of antiprotozoan drugs long used for the treatment of malaria; interferes with heme detoxification
- **quorum sensing** cell-to-cell communication in bacteria; enables a coordinated response from cells when the population reaches a threshold density

R

- **R plasmid** plasmid containing genes encoding proteins that make a bacterial cell resistant to one or more antibiotics
- **rabies** contagious viral disease primarily transmitted by the bite of infected mammals that can cause acute encephalitis resulting in madness, aggressiveness, coma, and death
- **radial immunodiffusion** precipitin reaction in which antigen added to a well in an antiserum-impregnated gel diffuses, producing a precipitin ring whose diameter squared is directly proportional to antigen concentration
- **rat-bite fever** relapsing fever caused by either *Bacillus moniliformis* or *Spirillum minor*; can be transmitted by the bite of a rat or through contact with rat feces or urine
- **reaction center** protein complex in a photosystem, containing a pigment molecule that can undergo oxidation upon excitation by a light-harvesting pigment, actually giving up an electron
- **reactivation tuberculosis** secondary infection by *Mycobacterium tuberculosis* that forms later in life; occurs when the bacteria escape from the Ghon complexes and establish focal infections at other sites in immunocompromised individuals
- **reactive oxygen species (ROS)** unstable and toxic ions and molecules derived from partial reduction of oxygen
- **reading frame** way nucleotides in mRNA are grouped into codons
- **real-time PCR (quantitative PCR, qPCR)** a variant of PCR involving the use of fluorescence to allow for the monitoring of the increase in double-stranded template during a PCR reaction as it occurs, allowing for the quantitation of the original target sequence
- **receptor-mediated endocytosis** a type of endocytosis in which extracellular ligands are targeted to specific cells through their binding

to specific cell surface receptors

- **recognition site** a specific, often palindromic, DNA sequence recognized by a restriction enzyme that is typically four to six base pairs long and reads the same in the 5' to 3' direction on one strand as it does in the 5' to 3' direction on the complementary strand
- **recombinant DNA molecule** a DNA molecule resulting from the cutting and insertion of DNA from one organism into the DNA of another organism, resulting in a new combination of genetic material
- **recombinant DNA pharmaceuticals** pharmaceuticals produced as a result of genetic engineering
- **recombinant DNA technology** the process by which DNA from one organism is cut and new pieces of foreign DNA from a second organism are inserted, artificially creating new combinations of genetic material within the organism
- **redox potential** tendency for a molecule to acquire electrons and become reduced; electrons flow from molecules with lower redox potentials to those with higher redox potentials
- **redox reaction** pairing of an oxidation reaction with a reduction reaction
- **reduction reaction** chemical reaction that adds electrons to acceptor molecules, leaving them reduced
- **reemerging infectious disease** a disease that was once under control or largely eradicated that has begun causing new outbreaks due to changes in susceptible populations, the environment, or the pathogen itself
- **reflection** when light bounces back from a surface
- **refraction** bending of light waves, which occurs when a light wave passes from one medium to another
- **refractive index** a measure of the magnitude of slowing of light waves by a particular medium
- **regulatory T cells** class of T cells that are activated by self-antigens and serve to inhibit peripheral self-reacting T cells from causing damage and autoimmunity
- **rejection** process by which adaptive immune responses recognize transplanted tissue as non-self, mounting a response that destroys the tissue or leads to the death of the individual

- **relapsing fever** louse- or tickborne disease caused by *Borrelia recurrentis* or *B. hermsii* and characterized by a recurrent fever
- **replica plating** plating technique in which cells from colonies growing on a complete medium are inoculated onto various types of minimal media using a piece of sterile velvet, ensuring that the orientation of cells deposited on all plates is the same so that growth (or absence thereof) can be compared between plates
- **replication bubble** circular structure formed when the DNA strands are separated for replication
- **replication fork** Y-shaped structure that forms during the process of replication as DNA unwinds and opens up to separate the DNA strands
- **replication** process by which DNA is copied
- **reporter genes** genes that encode easily observable characteristics, allowing for their expression to be easily monitored
- **repressible operon** bacterial operon, that typically containing genes encoding enzymes required for a biosynthetic pathway and that is expressed when the product of the pathway continues to be required but is repressed when the product of the pathway accumulates, removing the need for continued expression
- **repressor** protein that suppresses transcription of a gene or operon in response to an external stimulus
- **reservoir** a living host or nonliving site in which a pathogenic organism can survive or multiply
- **resident microbiota** microorganisms that constantly live in the human body
- **resolution** the ability to distinguish between two points in an image
- **restriction endonuclease (restriction enzyme)** bacterial enzyme that cuts DNA fragments at a unique, often palindromic, recognition site; used in genetic engineering for splicing DNA fragments together into recombinant molecules
- **restriction fragment length polymorphism (RFLP)** a genetic variant identified by differing numbers or sizes of DNA fragments generated after digestion of a DNA sample with a restriction endonuclease; the variants are caused by the loss or gain of restriction sites, or the insertion or deleting of sequences between restriction sites.
- **retort** large industrial autoclave used for moist heat sterilization on a large scale

- **retrospective study** a research design that associates historical data with present cases
- **retrovirus** positive ssRNA virus that produces and uses reverse transcriptase to make an ssDNA copy of the retroviral genome that can then be made into dsDNA and integrate into the host cell chromosome to form a provirus within the host chromosome.
- **reverse transcriptase** enzyme found in retroviruses that can make a copy of ssDNA from ssRNA
- **reverse transcriptase inhibitor** classes of antiviral drugs that involve nucleoside analog competitive inhibition and non-nucleoside noncompetitive inhibition of the HIV reverse transcriptase
- **reverse transcriptase PCR (RT-PCR)** a variation of PCR used to obtain DNA copies of a specific mRNA molecule that begins with the conversion of mRNA molecules to cDNA by the enzyme reverse transcriptase
- **Reye syndrome** potentially life-threatening sequelae to some viral infections that result in the swelling of the liver and brain; aspirin use has also been linked to this syndrome
- **Rh factor** red blood cell surface antigen that can trigger type II hypersensitivity reactions
- **rheostat** a dimmer switch that controls the intensity of the illuminator on a light microscope
- **rheumatic fever** serious clinical sequela of an infection with *Streptococcus pyogenes* that can result in damage to joints or the valves of the heart
- **rheumatoid arthritis** systemic autoimmune disease in which immune complexes form and deposit in the joints and their linings, leading to inflammation and destruction
- **rhinitis** inflammation of the nasal cavity
- **rhizines** structures made of hyphae found on some lichens; aid in attachment to a surface
- **ribonucleic acid (RNA)** single-stranded nucleic acid composed of ribonucleotides; important in transcription and translation (protein synthesis)
- **ribonucleotides** RNA nucleotides containing ribose as the pentose sugar component and a nitrogenous base
- **ribosome** a complex intracellular structure that synthesizes proteins

- **riboswitch** small region of noncoding RNA found within the 5' end of some prokaryotic mRNA molecules that may bind to a small intracellular molecule, influencing the completion of transcription and/or translation
- **ribulose bisphosphate carboxylase (RuBisCO)** first enzyme of the Calvin cycle responsible for adding a CO₂ molecule onto a five-carbon ribulose bisphosphate (RuBP) molecule
- **rifampin** semisynthetic member of the rifamycin class that blocks bacterial RNA polymerase activity, inhibiting transcription
- **rimantadine** antiviral drug that targets the influenza virus by preventing viral escape from endosomes upon host cell uptake, preventing viral RNA release and subsequent viral replication
- **ringworm** a tinea (cutaneous mycosis of the skin), typically characterized by a round, red, slightly raised lesion that heals outward from the center, giving it the appearance of a round worm
- **RNA interference (RNAi)** process by which antisense RNAs or small interfering RNAs (siRNAs) interfere with gene expression by binding to mRNA, preventing translation and protein synthesis
- **RNA polymerase** enzyme that adds nucleotides to the 3'-OH group of the growing mRNA molecule that are complementary to the template strand, forming covalent phosphodiester bonds between the nucleotides in the RNA
- **RNA splicing** process of removing intron-encoded RNA sequences from eukaryotic primary transcripts and reconnecting those encoded by exons
- **RNA transcript** mRNA produced during transcription
- **Rocky Mountain spotted fever** potentially fatal tickborne disease caused by *Rickettsia rickettsii* characterized by fever, body aches, and a rash
- **rogue form** misfolded form of the PrP protein that is normally found in the cell membrane and has the tendency to aggregate in neurons, causing extensive cell death and brain damage
- **rolling circle replication** type of rapid unidirectional DNA synthesis of a circular DNA molecule
- **roseola** a rash-causing illness, most commonly affecting children, associated with human herpesvirus 6 (HHV-6)

- **rough endoplasmic reticulum** a type of endoplasmic reticulum containing bound 80S ribosomes for the synthesis of proteins destined for the plasma membrane
- **route of administration** method used to introduce a drug into the body
- **rRNA** type of stable RNA that is a major constituent of ribosomes, ensuring proper alignment of the mRNA and the ribosomes as well as catalyzing the formation of the peptide bonds between two aligned amino acids during protein synthesis
- **rubella** German measles, caused by the rubella virus
- **runs (running)** purposeful, directional movement of a prokaryotic cell propelled by counterclockwise flagellar rotation

S

- **σ factor** subunit of bacterial RNA polymerase conferring promoter specificity that can be substituted with a different version in response to an environmental condition, allowing for a quick and global change of the regulon transcribed
- **saccharide** carbohydrate
- **salmonellosis** gastrointestinal illness caused by *Salmonella* bacteria
- **salpingitis** inflammation of the fallopian tubes
- **sandwich ELISA** EIA in which the primary antibody is first attached to the wells of a microtiter plate, allowing it to capture antigen from an unknown solution to be quantified
- **Sanger DNA sequencing (dideoxy method, chain termination method)** the original DNA sequencing technique in which dideoxy nucleotides, each labeled with a molecular beacon, are used to terminate chain elongation; the resulting incrementally sized fragments are then separated by electrophoresis to determine the sequence of the DNA molecule
- **sanitization** protocol that reduces microbial load on inanimate surfaces to levels deemed safe for public health
- **saprozoic** refers to protozoans that ingest small, soluble food molecules

- **SARS** severe acute respiratory syndrome; caused by a zoonotic coronavirus that results in flu-like symptoms
- **saturated fatty acid** lipid with hydrocarbon chains containing only single bonds, which results in the maximum number of hydrogen atoms per chain
- **scanning electron microscope (SEM)** a type of electron microscope that bounces electrons off of the specimen, forming an image of the surface
- **scanning probe microscope** a microscope that uses a probe that travels across the surface of a specimen at a constant distance while the current, which is sensitive to the size of the gap, is measured
- **scanning tunneling microscope** a microscope that uses a probe that is passed just above the specimen as a constant voltage bias creates the potential for an electric current between the probe and the specimen
- **scarlet fever** bacterial infection caused by *Streptococcus pyogenes*, marked by a high fever and a disseminated scarlet rash
- **schistosomiasis** helminthic infection caused by *Schistosoma* spp.; transmitted from a snail intermediate host to human swimmers or bathers in freshwater
- **schizogony** asexual reproduction in protozoans that is characterized by multiple cell divisions (one cell dividing to form many smaller cells)
- **scolex** the head region of a cestode (tapeworm), which typically has suckers and/or hooks for attachment to the host
- **scrapie** form of transmissible spongiform encephalopathy that primarily affects sheep
- **sebaceous gland** a gland located in hair follicles that secretes sebum
- **sebum** lipid-rich substance secreted by the sebaceous glands of the skin
- **secondary antibody** antibody to which an enzyme is attached for use in ELISA assays; in direct and sandwich ELISAs, it is specific for the antigen being quantified, whereas in indirect ELISA, it is specific for the primary antibody
- **secondary immunodeficiency** impaired immune response due to infection, metabolic disturbance, poor diet, stress, or other acquired factors
- **secondary infection** second infection that develops after a primary infection as a result of the primary disease compromising immune

defenses or antibiotics, thus eliminating protective microbiota

- **secondary lymphoid tissue** one of two types of lymphatic tissue; comprises the spleen, lymph nodes, Peyer's patches, and mucosa associated lymphoid tissue (MALT)
- **secondary response** the adaptive immune response produced in response to a specific antigen to which the body has previously been exposed
- **secondary structure** structure stabilized by hydrogen bonds between the carbonyl and amine groups of a polypeptide chain; may be an α -helix or a β -pleated sheet, or both
- **secretory vesicle** membranous sac that carries molecules through the plasma membrane to be released (secreted) from the cell
- **selective IgA deficiency** primary immunodeficiency in which individuals produce normal levels of IgG and IgM, but are unable to produce secretory IgA
- **selective media** media that contain additives that encourage the growth of some bacteria while inhibiting others
- **selective toxicity** desirable quality of an antimicrobial drug indicating that it preferentially kills or inhibits the growth of the target microbe while causing minimal or no harm to the host
- **semiconservative DNA replication** pattern of DNA replication process whereby each of the two parental DNA strands acts as a template for new DNA to be synthesized, producing hybrid old- and new-strand daughter molecules
- **semicritical item** object that contacts mucous membranes or nonintact skin but does not penetrate tissues; requires a high level of disinfection
- **seminal vesicles** glands that contribute fluid to semen
- **semisynthetic antimicrobial** chemically modified derivative of a natural antibiotic
- **sense strand** strand of DNA that is not transcribed for gene expression; it is complementary to the antisense strand
- **sepsis** systemic inflammatory response to an infection that results in high fever and edema, causing organ damage and possibly leading to shock and death
- **septate hyphae** hyphae that contain walls between individual cells; characteristic of some fungi
- **septic arthritis** see *infectious arthritis*

- **septic shock** serious condition marked by the loss of blood pressure resulting from an inflammatory response against a systemic infection
- **septic** the condition of being septicemic; having an infection in the blood
- **septicemia** condition in which pathogens are multiplying in blood
- **septicemic plague** form of plague that occurs when the bacterial pathogen gains access to the bloodstream
- **septum** separating structure that forms during cell division; also describes the separating wall between cells in a filament
- **sequela (plural: sequelae)** condition that arises as a consequence of a prior disease
- **serial dilution** sequential transfer of known volumes of culture samples from one tube to another to perform a several-fold dilution of the original culture
- **seroconversion** point in an infection at which antibody to a pathogen is detectable using an immunoassay
- **serotype** strain or variation of the same species of bacteria; also called serovar
- **serovar** specific strain of bacteria identified by agglutination using strain-specific antisera
- **serum** fluid portion of the blood after clotting has occurred; generally lacks clotting factors
- **serum sickness** systemic type III hypersensitivity reaction
- **sessile** attached to a surface
- **severe combined immunodeficiency disease (SCID)** genetic disorder resulting in impaired function of B cells and T cells
- **sex pilus** specialized type of pilus that aids in DNA transfer between some prokaryotic cells
- **sheath** part of the tail on a bacteriophage that contracts to introduce the viral DNA into the bacterium
- **shigellosis** gastrointestinal illness caused by *Shigella* bacteria, also called bacillary dysentery
- **shingles** acute and painful rash that forms following the reactivation of a latent chickenpox infection
- **shock** extreme drop in blood pressure that, among other causes, can result from a strong immune response to the activity of toxins or response to bacterial products and can result in death

- **shuttle vector** a plasmid that can move between bacterial and eukaryotic cells
- **side chain** the variable functional group, R , attached to the α carbon of an amino acid
- **sign** objective and measurable indication of a disease
- **silent mutation** point mutation that results in the same amino acid being incorporated into the resulting polypeptide
- **simple microscope** a type of microscope with only one lens to focus light from the specimen
- **simple staining** a staining technique that uses a single dye
- **single-stranded binding protein** protein that coats the single strands of DNA near each replication fork to prevent the single-stranded DNA from rewinding into a double helix
- **sinusitis** inflammation of the sinuses
- **S-layer** cell envelope layer composed of protein covering the cell walls of some bacteria and archaea; in some archaea, may function as the cell wall
- **slime layer** a type of glycocalyx with unorganized layers of polysaccharides that aid bacterial adherence to surfaces
- **smear** a thin layer of a specimen on a slide
- **smooth endoplasmic reticulum** a type of endoplasmic reticulum that lacks ribosomes, is involved in the biosynthesis of lipids and in carbohydrate metabolism, and serves as the site of detoxification of toxic compounds within the cell
- **soft chancres** soft, painful ulcers associated with the STI chancroid
- **soma** cell body of a neuron
- **sonication** method of microbial control that involves application of ultrasound waves to form cavitation within a solution, including inside cells, disrupting cell components as a result
- **Southern blot** a technique in molecular genetics used to detect the presence of certain DNA sequences within a given DNA sample; DNA fragments within the sample are separated by agarose gel electrophoresis, immobilized on a membrane, and then exposed to a specific DNA probe labeled with a radioactive or fluorescent molecular beacon to aid in detection
- **specialized transduction** transfer of a specific piece of bacterial chromosomal DNA near the site of integration by the phage

- **specificity** the ability of the specific adaptive immune system to target specific pathogens or toxins
- **spike** viral glycoprotein embedded within the viral capsid or envelope used for attachment to host cells
- **spirochetes** a group of long, thin, spiral-shaped fastidious bacteria that includes the human pathogens that cause syphilis, Lyme disease, and leptospirosis
- **spleen** abdominal organ consisting of secondary lymphoid tissue that filters blood and captures pathogens and antigens that pass into it; also contains specialized macrophages and dendritic cells that are crucial for antigen presentation
- **spliceosome** protein complex containing small nuclear ribonucleoproteins that catalyzes the splicing out of intron-encoded RNA sequences from the primary transcript during RNA maturation in eukaryotes
- **spontaneous generation** the now-disproven theory that life can arise from nonliving matter
- **spontaneous mutation** mutation not caused by a mutagen that occurs through DNA replication errors
- **sporadic disease** an illness that occurs at relatively low levels with no discernible pattern or trend, frequently with no geographic focus
- **spores** specialized cells that may be used for reproduction or may be specialized to withstand harsh conditions
- **sporotrichosis** subcutaneous infection caused by the fungus *Sporothrix schenkii*, which causes skin lesions and can potentially spread to the lymphatic system; also known as rose gardener's disease or rose thorn disease
- **sporulation** the process by which a vegetative cell produces a dormant endospore
- **spread plate method** a technique used for inoculating plates with diluted bacterial samples for the purpose of cell counting; the liquid sample is pipetted onto solid medium and spread uniformly across the plate
- **St. Louis encephalitis** mosquito-borne viral infection of the brain that occurs primarily in the central and southern United States
- **stage** the platform of a microscope on which slides are placed

- **staining** the addition of stains or dyes to a microscopic specimen for the purpose of enhancing contrast
- **staphylococcal food poisoning** gastrointestinal illness caused by toxins produced by *Staphylococcus aureus*
- **staphylolysins** a class of staphylococcal exotoxins that are cytotoxic to skin cells and white blood cells
- **starch** energy-storage polysaccharide in plants; composed of two types of glucose polymers: amylose and amylopectin
- **start codon** AUG codon, specifying methionine, which is typically the codon that initiates translation
- **stationary phase** interval during which the number of cells formed by cell division is equal to the number of cells dying
- **stereoisomers** isomers that differ in the spatial arrangements of atoms
- **sterilant** strong chemical that effectively kills all microbes and viruses in or on an inanimate item
- **sterile field** specified area that is free of all vegetative microbes, endospores, and viruses
- **sterilization** protocol that completely removes all vegetative cells, endospores, and viruses from an item
- **steroid** lipid with complex, ringed structures found in cell membranes and hormones
- **sterol** the most common type of steroid; contains an OH group at one specific position on one of the molecule's carbon rings
- **sticky ends** short, single-stranded complementary overhangs that may be produced when many restriction enzymes cut DNA
- **stigma** light-sensing eyespot found in *Euglena*
- **stop codon (nonsense codon)** one of three codons for which there is no tRNA with a complementary anticodon; a signal within the mRNA for termination of translation
- **stratum corneum** a layer of dead, keratinized cells that forms the uppermost layer of the epidermis
- **strep throat (streptococcal pharyngitis)** bacterial pharyngitis caused by *Streptococcus pyogenes*
- **streptococcal toxic shock-like syndrome (STSS)** condition similar to staphylococcal toxic shock syndrome but with greater likelihood of bacteremia, necrotizing fasciitis, and acute respiratory distress syndrome

- **stroma** a gel-like fluid that makes up much of a chloroplast's volume, and in which the thylakoids floats
- **strongyloidiasis** soil-transmitted intestinal infection caused by the helminth *Strongyloides stercoralis*
- **structural formula** graphic representation of the molecular structure showing how the atoms are arranged
- **structural isomers** molecules composed of the same numbers and types of atoms but with different bonding sequences
- **subacute bacterial endocarditis** form of endocarditis in which damage to the valves of the heart occurs over months as a result of blood clot formation and immune-response-induced fibrosis of the valves
- **subclinical disease** disease that does not present any signs or symptoms
- **subcutaneous mycosis** any fungal infection that penetrates the epidermis and dermis to enter deeper tissues
- **substrate** chemical reactants of an enzymatic reaction
- **substrate-level phosphorylation** direct method of ATP production in which a high-energy phosphate group is removed from an organic molecule and added to an ADP molecule
- **subunit vaccine** vaccine that contains only key antigens as opposed to whole pathogens
- **sugar-phosphate backbone** alternating sugar-phosphate structure composing the framework of a nucleic acid strand that results from phosphodiester bond formation between nucleotides
- **sulfonamides (sulfa drugs)** group of structurally related synthetic antimicrobial compounds that function as antimetabolites, competitively inhibiting an enzyme in the bacterial folic acid synthesis pathway
- **superantigen** class of exotoxin that triggers a strong nonspecific immune response with excessive production of cytokines (cytokine storm) causing inflammation, high fever, shock, and, potentially, death
- **supercoiled** extensive wrapping and twisting of a DNA molecule, allowing the DNA to fit within a small space
- **supercoiling** process in which DNA is underwound or overwound to fit inside a cell

- **supercritical fluid** molecule, commonly carbon dioxide, brought to high pressures to reach a state that has physical properties between those of liquids and gases, allowing it to effectively penetrate surfaces and cells to form carbonic acid, which lowers the pH of cells considerably, leading to sterilization
- **superinfection** secondary infection that may develop as a result of long-term, broad-spectrum antimicrobial use
- **superoxide dismutase** enzyme that catalyzes the breakdown of superoxide anions
- **suppurative** producing pus; purulent
- **surfactant** group of chemical compounds used for degerning; lower the surface tension of water, creating emulsions that mechanically carry away microorganisms
- **sweat gland** one of numerous tubular glands embedded in the dermis that secretes the watery substance known as perspiration
- **symbiosis** any interaction between different species that are associated with each other within a community
- **symptom** subjective experience of disease felt by the patient
- **synapse** junction between a neuron and another cell
- **syncytia** multinucleated cells that form from the fusion of normal cells during infections or other processes
- **syndrome** group of signs and symptoms characteristic of a particular disease
- **syngamy** process in which haploid gametes fuse
- **synthetic antimicrobial** antimicrobial developed from a chemical not found in nature
- **syphilis** an STI caused by the bacterium *Treponema pallidum*
- **systemic autoimmune disease** autoimmune disease that affect the organism as a whole, rather than a single organ
- **systemic infection** infection that has spread to multiple locations or body systems
- **systemic inflammatory response syndrome (SIRS)** severe inflammatory response to the presence of microbes in the blood; can lead to sepsis
- **systemic lupus erythematosus (SLE)** systemic autoimmune disease producing inflammatory type III hypersensitivities as antibodies form immune complexes with nuclear and cytoplasmic antigens

- **systemic mycosis** a fungal infection that spreads throughout the body

T

- **T-cell receptors (TCR)** molecules on T cells involved in the recognition of processed foreign epitopes presented with MHC I or MHC II
- **T lymphocyte** lymphocyte that serves as the central orchestrator, bridging humoral, cellular, and innate immunity, and serves as the effector cells of cellular immunity; T cell
- **taeniasis** infection caused by *Taenia* or *Diphyllobothrium*
- **tail fiber** long protein component on the lower part of a phage used for specific attachment to bacterial cell
- **tail pins** points extended at the base of a bacteriophage sheath that, along with tail fibers, lead to phage attachment to a bacterial cell
- **tapeworms** segmented, hermaphroditic, parasitic flatworms (Platyhelminthes)
- **tartar** calcified heavy plaque on teeth, also called dental calculus
- **TDP** thermal death point is the lowest temperature at which all microorganisms are killed in a 10-minute exposure
- **TDT** thermal death time is the length of time needed to kill all microorganisms in a sample at a given temperature
- **telomerase** enzyme that attaches to the end of a linear chromosome and adds nucleotides to the 3' end of one of the DNA strands, maintaining the telomere sequence, thus preventing loss of DNA from the end of the chromosome
- **telomere** repetitive, noncoding sequence found at the end of a linear eukaryotic chromosome that protects the genes near the end of the chromosome from deletion as the DNA molecule is repeatedly replicated
- **temperate phage** bacteriophage that can incorporate viral genome into the host cell chromosome and replicate with the host cell until new

viruses are produced; a phage that undergoes the lysogenic cycle

- **teratogenic** able to disrupt the normal development of a fetus in utero
- **terbinafine** antifungal drug of the allylamine class that is used topically for the treatment of dermatophytic skin infections
- **termination of DNA replication** stage of replication during which DNA replication is halted once the chromosome has been fully replicated
- **termination of transcription** stage of transcription that occurs when RNA polymerase has reached specific DNA sequences, leading to release of the enzyme from the DNA template, freeing the RNA transcript and, thus, halting transcription
- **termination of translation** stage of translation during which a nonsense codon aligns with the A site, signaling release factors to release of the polypeptide, leading to the dissociation of the small and large ribosomal subunits from the mRNA and from each other
- **tertiary structure** large-scale, three-dimensional structure of a polypeptide
- **test sensitivity** probability that a diagnostic test will find evidence of the targeted disease when the pathogen is present
- **test specificity** probability that a diagnostic test will not find evidence of the targeted disease when the pathogen is absent
- **testes** (singular *testis*) pair of glands located in the scrotum of males that produce sperm and testosterone
- **tetanus** bacterial disease caused by exotoxin produced by *Clostridium tetani* that causes a rigid paralysis
- **tetracyclines** class of protein synthesis inhibitors that bind to the 30S subunit, blocking the association of tRNAs with the ribosome during translation
- **T_H1 cells** subtype of T cells that stimulate cytotoxic T cells, macrophages, neutrophils, and NK cells
- **T_H17 cells** subtype of T cell that are essential for defense against specific pathogens and infections, such as chronic mucocutaneous infections with *C. albicans*
- **T_H2 cells** subtype of T cells that stimulate B cells and direct their differentiation; also involved in directing antibody class switching
- **thallus** body of fleshy fungi (more generally, a body without a root, stem, or leaf) that commonly co-occurs with HIV infection; the

microbes move to the lymphatic system in the groin

- **thermophile** a microorganism that grows best at warm temperatures, typically between about 50 °C and 80 °C
- **thin sections** thin slices of tissue for examination under a TEM
- **thioglycolate medium** medium designed to test the aerotolerance of bacteria; it contains a low concentration of agar to allow motile bacteria to move throughout the medium
- **thioglycolate tube culture** contains reducing medium through which oxygen diffuses from the tube opening, producing a range of oxygen environments down the length of the tube
- **thrombocytes** see *platelets*
- **thylakoids** a highly dynamic collection of membranous sacs found in the stroma of chloroplasts; site of photosynthesis
- **thymic selection** a three-step process of negative and positive selection of T cells in the thymus
- **thymine dimer** covalent linkage between two adjacent thymine bases on exposure to ultraviolet radiation
- **thymine** pyrimidine nitrogenous base found only in DNA nucleotides
- **tincture** solution of an antiseptic compound dissolved in alcohol
- **T-independent antigen** a nonprotein antigen that can activate a B cell without cooperation from a helper T cell
- **tinea** any cutaneous fungal infection caused by dermatophytes, such as tinea corporis, tinea capitis, tinea cruris, and tinea pedis
- **tinea capitis** cutaneous mycosis of the scalp; also known as ringworm of the scalp
- **tinea corporis** cutaneous mycosis of the body; also known as ringworm of the body
- **tinea cruris** cutaneous mycosis of the groin region; also known as jock itch
- **tinea pedis** cutaneous mycosis of the feet; also known as athlete's foot
- **tissue tropism** tendency of most viruses to infect only certain tissue types within a host
- **titer** concentration obtained by titration; the reciprocal of a measurement of biological activity determined by finding the dilution of an unknown (e.g., antigen-specific antibody in an antiserum) that shows the defined end-point; always expressed as a whole number
- **tolerance** lack of an anti-self immune response

- **toll-like receptors (TLRs)** pathogen recognition receptors (PRRs) that may be found on the external surface of phagocytes or facing inward in interior compartments
- **tonsillitis** inflammation of the tonsils
- **topoisomerase** type of enzyme that helps maintain the structure of supercoiled chromosomes, preventing overwinding of DNA during certain cellular processes like DNA replication
- **topoisomerase II** enzyme responsible for facilitating topological transitions of DNA, relaxing it from its supercoiled state
- **total magnification** in a light microscope is a value calculated by multiplying the magnification of the ocular by the magnification of the objective lenses
- **toxemia** presence of toxins in the blood
- **toxic shock syndrome** severe condition marked by the loss of blood pressure and blood clot formation caused by a bacterial superantigen, toxic shock syndrome toxin
- **toxigenicity** ability of a pathogen to produce toxins to cause damage to host cells
- **toxin** poison produced by a pathogen
- **toxoid vaccine** vaccine that contains inactivated bacterial toxins
- **toxoplasmosis** typically asymptomatic protozoan infection caused by *Toxoplasma* spp. and transmitted through contact with cysts in cat feces; infections in pregnant women may cause birth defects or miscarriage
- **trace element** indispensable element present in cells in lower amounts than macronutrients; also called *micronutrient*
- **trachea** also known as the windpipe, this is a stiffened tube of cartilage that runs from the larynx to the bronchi
- **trachoma** a type of conjunctivitis, caused by *Chlamydia trachomatis*, that is a major cause of preventable blindness
- **transcription bubble** region of unwinding of the DNA double helix during transcription
- **transcription factors** proteins encoded by regulatory genes that function by influencing the binding of RNA polymerase to the promoter and allowing its progression to transcribe structural genes
- **transcription** process of synthesizing RNA using the information encoded in DNA

- **transcriptomics** the study of the entire collection of mRNA molecules produced by cells; involves monitoring differences in gene expression patterns between cells at the mRNA level
- **transduction** mechanism of horizontal gene transfer in bacteria in which genes are transferred through viral infection
- **transendothelial migration** process by which circulating leukocytes exit the bloodstream via the microvascular endothelium
- **transfection** the introduction of recombinant DNA molecules into eukaryotic hosts
- **transformation** mechanism of horizontal gene transfer in bacteria in which naked environmental DNA is taken up by a bacterial cell
- **transgenic** describing an organism into which foreign DNA from a different species has been introduced
- **transient microbiota** microorganisms, sometimes pathogenic, that are only temporarily found in the human body
- **transition reaction** reaction linking glycolysis to the Krebs cycle, during which each pyruvate is decarboxylated and oxidized (forming NADH), and the resulting two-carbon acetyl group is attached to a large carrier molecule called coenzyme A, resulting in the formation of acetyl-CoA and CO; also called the *bridge reaction*
- **translation (protein synthesis)** process of protein synthesis whereby a ribosome decodes an mRNA message into a polypeptide product
- **transmissible spongiform encephalopathy** degenerative disease caused by prions; leads to the death of neurons in the brain
- **transmission electron microscope (TEM)** a type of electron microscope that uses an electron beam, focused with magnets, that passes through a thin specimen
- **transmittance** the amount of light that passes through a medium
- **transparency** the property of allowing light to pass through
- **transport vesicle** membranous sac that carries molecules between various components of the endomembrane system
- **transposition** process whereby a DNA sequence known as a transposon independently excises from one location in a DNA molecule and integrates elsewhere
- **transposon (transposable element)** molecule of DNA that can independently excise from one location in a DNA molecule and integrate into the DNA elsewhere

- **trench fever** louseborne disease caused by *Bartonella quintana* and characterized by high fever, body aches, conjunctivitis, ocular pain, severe headaches, and severe bone pain
- **trench mouth** a severe form of gingivitis, also called acute necrotizing ulcerative gingivitis
- **treponemal serologic tests** tests for syphilis that measure the amount of antibody directed against antigens associated with *Treponema pallidum*
- **triacylglycerol** three fatty acids chemically linked to a glycerol molecule; also called a triglyceride
- **triazoles** ergosterol biosynthesis inhibitors used to treat several types of systemic yeast infections; exhibit more selective toxicity than the imidazoles and are associated with fewer side effects
- **tricarboxylic acid cycle** see *Krebs cycle*
- **trichinosis** soil-transmitted intestinal infection caused by the nematode *Trichinella spiralis*; associated with cyst formation
- **trichomoniasis** a common STI caused by *Trichomonas vaginalis*
- **trichuriasis** intestinal infection caused by the whipworm *Trichuris trichiura*
- **triglyceride** three fatty acids chemically linked to a glycerol molecule; also called a triacylglycerol
- **trimethoprim** synthetic antimicrobial compound that functions as an antimetabolite to an enzyme in the bacterial folic acid synthesis pathway
- **tRNA** small type of stable RNA that carries the correct amino acid to the site of protein synthesis in the ribosome and base pairs with the mRNA to allow the amino acid it carries to be inserted in the polypeptide chain being synthesized
- **trophozoite** a life cycle phase in which protists are actively feeding and growing
- **tubercle** small, rounded lesion
- **tuberculosis** life-threatening form of microbial infection marked by the presence of acid-fast bacteria growing in nodules (especially in the lungs)
- **tularemia** infection of the lymphatic system by *Francisella tularensis*; also known as rabbit fever

- **tumbles (tumbling)** random, circuitous movement of a bacterial cell, propelled by clockwise flagellar rotation
- **tumor** collection or aggregate of cells; can be benign (noncancerous) or malignant (cancerous)
- **tumor-inducing (T_i) plasmid** a naturally occurring plasmid of the bacterium *Agrobacterium tumefaciens* that researchers use as a shuttle vector to introduce a desired DNA fragment into plant cells
- **turbidity** cloudiness of a culture due to refraction of light by cells and particles
- **two-photon microscope** a microscope that uses long-wavelength or infrared light to fluoresce fluorochromes in the specimen
- **tympanic membrane** also referred to as the ear drum, this structure separates the outer and middle ear
- **type 1 diabetes mellitus** hyperglycemia caused by an autoimmune disease affecting insulin production by β cells of the pancreas
- **type I hypersensitivity** rapid-onset allergic reaction due to cross-linking of antigen-specific IgE on the outside of mast cells, resulting in release of inflammatory mediators
- **type II hypersensitivity** cytotoxic reaction triggered by IgG and IgM antibodies binding to antigens on cell surfaces
- **type III hypersensitivity** inflammatory reaction induced by formation of immune complexes and their deposition in tissues and blood vessels
- **type IV hypersensitivity** delayed T-cell-mediated inflammatory reaction that takes longer to manifest than the first three hypersensitivity types, due to the need for activation of antigen-presenting cell and T-cell subsets
- **typhoid fever** serious illness caused by infection with certain serotypes of *Salmonella*

U

- **UHT pasteurization** method of pasteurization that exposes milk to ultra-high temperatures (near 140 °C) for a few seconds, effectively sterilizing it so that it can be sealed and stored for long periods without refrigeration
- **ulcer** open sore

- **ultramicrotome** a device that cuts thin sections for electron microscopy
- **unit membrane** biological membrane composed of two layers of phospholipid molecules with the nonpolar tails associating to form a hydrophobic barrier between the polar heads; also called lipid bilayer
- **unsaturated fatty acid** lipid with hydrocarbon chains containing one or more carbon-carbon double bonds and subsequently fewer than the maximum number of hydrogen atoms per chain
- **uracil** pyrimidine nitrogenous base found only in RNA nucleotides
- **ureter** duct that transports urine from the kidneys to the urinary bladder
- **ureteritis** inflammation of the ureter
- **urethra** duct through which urine passes from the urinary bladder to leave the body through the urinary meatus
- **urethritis** inflammation of the urethra
- **urinary bladder** an organ that stores urine until it is ready to be excreted
- **urinary meatus** the opening through which urine leaves the body
- **use-dilution test** a technique for determining the effectiveness of a chemical disinfectant on a surface; involves dipping a surface in a culture of the targeted microorganism, disinfecting the surface, and then transferring the surface to a fresh medium to see if bacteria will grow
- **uterus** female reproductive organ in which a fertilized egg implants and develops

V

- **vaccination** inoculation of a patient with attenuated pathogens or antigens to activate adaptive immunity and protect against infection
- **vagina** female reproductive organ that extends from the vulva to the cervix
- **vaginitis** inflammation of the vagina
- **vaginosis** an infection of the vagina caused by overgrowth of resident bacteria
- **vancomycin** cell wall synthesis inhibitor of the glycopeptide class

- **vancomycin-intermediate *Staphylococcus aureus* (VISA)** pathogen with intermediate vancomycin resistance due to increased targets for and trapping of vancomycin in the outer cell wall
- **vancomycin-resistant enterococci (VRE)** pathogens resistant to vancomycin through a target modification of peptidoglycan subunit peptides that inhibit binding by vancomycin
- **vancomycin-resistant *Staphylococcus aureus* (VRSA)** pathogen with resistance to vancomycin that has arisen as a result of the horizontal gene transfer of vancomycin resistance genes from VRE
- **variolation** the historical practice of inoculating a healthy patient with infectious material from a person infected with smallpox in order to promote immunity to the disease
- **vas deferens** pair of ducts in the male reproductive system that conduct sperm from the testes and seminal fluid to the ejaculatory duct
- **vasculitis** inflammation affecting blood vessels (either arteries or veins)
- **VDRL (Venereal Disease Research Laboratory) test** test for syphilis that detects anti-treponemal antibodies to the phospholipids produced due to the tissue destruction by *Treponema pallidum*; antibodies are detected through a flocculation reaction with cardiolipin extracted from beef heart tissue
- **vector** animal (typically an arthropod) that transmits a pathogen from one host to another host; DNA molecules that carry DNA fragments from one organism to another
- **vegetative cell** a cell that is actively growing and dividing, and does not contain an endospore
- **vehicle transmission** transfer of a pathogen between hosts via contaminated food, water, or air
- **vein** blood vessel that returns blood from the tissues to the heart for recirculation
- **vertical direct transmission** transfer of a pathogen from mother to child during pregnancy, birth, or breastfeeding
- **vertical gene transfer** transfer of genes from parent to offspring
- **viable cell** live cell; live cells are usually detected as colony-forming units
- **viable plate count** direct method of measuring microbial growth in a culture; the number of viable or live cells is usually expressed in

CFU/mL

- **viral conjunctivitis** inflammation of the conjunctiva caused by a viral infection
- **viral envelope** lipid membrane obtained from phospholipid membranes of the cell that surrounds the capsid
- **viral hemagglutination inhibition assay** assay used to quantify the amount of neutralizing antibody against a virus by showing a decrease in hemagglutination caused by a standardized amount of virus
- **viral titer** number of virions per unit volume
- **viremia** presence of virus in blood
- **viricide** chemical or physical treatment that destroys or inactivates viruses
- **virion** inert particle that is the reproductive form of a virus
- **viroid** infectious plant pathogen composed of RNA
- **virology** the study of viruses
- **virulence** degree to which an organism is pathogenic; severity of disease signs and symptoms
- **virulence factor** product of a pathogen that assists in its ability to cause infection and disease
- **virulent phage** bacteriophage for which infection leads to the death of the host cell; a phage that undergoes the lytic cycle
- **virus** an acellular microorganism, consisting of proteins and genetic material (DNA or RNA), that can replicate itself by infecting a host cell
- **virusoid** small piece of RNA associated with larger RNA of some infectious plant viruses
- **volutin** inclusions of polymerized inorganic phosphate; also called metachromatic granules
- **vulva** the female external genitalia

W

- **water activity** water content of foods or other materials
- **wavelength** the distance between one peak of a wave and the next peak

- **Weil's disease** advanced stage of leptospirosis in which the kidney and liver become seriously infected
- **West African trypanosomiasis** chronic form of African trypanosomiasis caused by *Trypanosoma brucei gambiense*
- **West Nile encephalitis** mosquito-borne disease caused by the West Nile virus (WNV) that can result in swelling of the brain and death in severe cases
- **western blot** technique used to detect the presence of a certain protein within a given protein sample in which proteins within the sample are separated by PAGE, immobilized on a membrane, and then exposed first to an antibody that binds to the protein of interest and then second to an antibody equipped with a molecular beacon that will bind to the first antibody
- **western equine encephalitis** serious but rare mosquito-borne viral infection of the brain that is found primarily in the central and western United States
- **wet mount** a slide preparation technique in which a specimen is placed on the slide in a drop of liquid
- **wheal-flare reaction** localized type I hypersensitivity reaction, involving a raised, itchy bump (wheal) and redness (flare), to injected allergen
- **whooping cough** common name for pertussis
- **wild type** phenotype of an organism that is most commonly observed in nature
- **Winterbottom's sign** acute swelling of lymph nodes at the back of the neck that is an early sign of African trypanosomiasis
- **wobble position** third position of a codon that, when changed, typically results in the incorporation of the same amino acid because of the degeneracy of the genetic code
- **World Health Organization (WHO)** international public health organization within the United Nations; monitors and communicates international public health information and coordinates international public health programs and emergency interventions

- **xenobiotic** compound synthesized by humans and introduced to an environment in much higher concentrations than expected in nature
- **xenograft** transplanted tissue from a donor that is of a different species than the recipient
- **X-linked agammaglobulinemia** genetic disorder resulting in an inability to produce antibodies
- **x-y mechanical stage knobs** knobs on a microscope that are used to adjust the position of the specimen on the stage surface, generally to center it directly above the light

Y

- **yeast** any unicellular fungus
- **yeast infection** fungal infection of the vagina typically caused by an overgrowth of resident *Candida* spp.
- **yellow fever** mild to potentially fatal mosquito-borne viral disease caused by the yellow fever virus

Z

- **Ziehl-Neelsen technique** a method of acid-fast staining that uses heat to infuse the primary stain, carbolfuchsin, into acid-fast cells
- **zone of inhibition** clear zone around a filter disk impregnated with an antimicrobial drug, indicating growth inhibition due to the antimicrobial drug
- **zoonosis** see *zoonotic disease*
- **zoonotic disease** any disease that is transmitted to humans by animals
- **zooplankton** heterotrophic plankton
- **Z-scheme** electron flow seen in noncyclic photophosphorylation in plants, algae, and cyanobacteria due to the use of both PSI and PSII
- **zygospores** spores used by Zygomycetes for sexual reproduction; they have hard walls formed from the fusion of reproductive cells from two individuals